Please Direct All Correspondence to Customer Number **20995**

TRANSMITTAL LETTER
APPEAL BRIEF

Applicant	:	Paul P. Latta
App. No	:	10/823,263
Filed	:	April 13, 2004
For	:	A METHOD OF TREATMENT OF DIABETES THROUGH INDUCTION OF IMMUNOLOGICAL TOLERANCE
Examiner	:	Belyavakyi, Michail
Art Unit	:	1644

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Mai Stop Appeal Brief – Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

April 7, 2006
(Date)

Daniel E. Altman, Reg. No. 34,115

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith for filing in the above-identified application are the following enclosures:

(X) Appeal Brief in 13 pages.

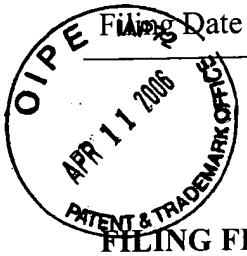
(X) 10 Appendices:

1. Specification as filed;
2. Office Action, mailed January 26, 2005;
3. Office Action, mailed June 16, 2005;
4. Final Office Action, mailed November 3, 2005;
5. USP 6,703,017;
6. USP 5,425,764;
7. USP 5,629,194;
8. Posselt et al. 1991 "Intrathymic islet transplantation in BB rats" Ann. Surg. **214**:363-373;
9. Response to Office Action, filed May 6, 2005

Docket No. : LATTA.002C4 **Customer No.: 20,995**

Application No. : 10/823,263

Filing Date : April 13, 2004



10. Dr. D. Scharp Declaration under 37 CFR §1.132, filed May 6, 2005.

FILING FEES:

The present application qualifies for Small Entity Status under 37 CFR 1.27.

FEE CALCULATION				
FEE TYPE		**FEE CODE**	**CALCULATION**	**TOTAL**
Appeal Brief	*41.20(b)(2)*	2402 ($250)		$250
1 Month Extension	*1.17(a)(1)*	2251 ($60)		$
2 Month Extension	*1.17(a)(2)*	2252 ($225)		$
3 Month Extension	*1.17(a)(3)*	2253 ($510)		$
			TOTAL FEE DUE	$250

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Dated: April 7, 2006

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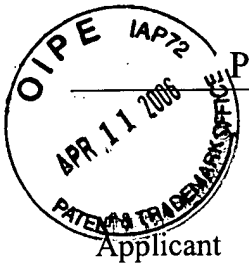

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Art Unit	:	1644

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the Notice of Appeal filed February 3, 2006, Applicant submits this Appeal Brief.

TABLE OF CONTENTS

I. REAL PARTY IN INTEREST

Pursuant to 37 C.F.R. §1.192, Appellants hereby notify the Board of Patent Appeals and Interferences that the real party in interest is the inventor for this application, Paul P. Latta, 33 Santa Cruz Aisle, Irvine, CA 92606.

II. RELATED APPEALS AND INTERFERENCES

Appellants are currently appealing to the US Board of Patent Appeals and Interferences in a related case, No.: 10/660,924.

III. STATUS OF CLAIMS

The above-identified application was filed with 14 claims. In response to the Office Action mailed June 16, 2005, claim 12 was cancelled and Claims 1, 6, and 11 were amended. Claims 1-11, 13 and 14 were finally rejected by the Examiner in the Final Office Action mailed November 3, 2005. Accordingly, Claims 1-11, 13 and 14 are the subject of this appeal. The Claims are attached hereto as Appendix A.

IV. STATUS OF AMENDMENTS

No amendments have been filed subsequent to the final rejection.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter relates to Appellant's discovery that type I diabetes in a mammal can be treated by implanting into said mammal a tolerizing dose of insulin-producing cells encapsulated in a biologically-compatible membrane, followed by the second step of implanting a fully therapeutic dose of un-encapsulated insulin-producing cells, wherein the therapeutic dose of the insulin-producing cells at least one order of magnitude higher than the tolerizing dose. The encapsulated cells shed antigens through the capsule membrane into the blood stream of a host, while being protected from the attack by the host immune system. Over time, the host immune system gets tolerized to the implanted cells and when the fully therapeutic

dose of the same cells is implanted, it is recognized by the immune system as "self" and a rejection response is not elicited.[1].

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. The Examiner has rejected Claims 1-4, 6-11, 13 and 14 under 35 U.S.C. §103(a), as being obvious over USP 6,703,017, or by USP 5,425,764 or USP 5,629,194 each in view of Posselt et al. (*Diabetes*, 1992, **41**:771-775)[2].

2. The Examiner has also rejected Claim 5 under 35 U.S.C. §103(a), as being obvious over USP 6,703,017, or by USP 5,425,764 or USP 5,629,194 each in view of Posselt et al. (*Diabetes*, 1992, **41**:771-775) as applied to claims 1-4 and 6-14, and further in view of USP 5,529,914[3].

VII. ARGUMENT

1. The Examiner has improperly rejected Claims 1-4, 6-11, 13 and 14 as obvious.

> Pursuant to MPEP 2143, in order to establish a *prima facie* case of obviousness three requirements must be met: First there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

None of the three primary references cited by the Examiner provide any teaching of a tolerizing dose followed by a therapeutic dose. All three of these patents teach only the administration of a single fully-therapeutic dose. For example, U.S. Patent 6,703,017 describes treating diabetes in a mammal by creating a pancreas-like structure in a human patient. The patent indicates that "from data relating to transplantation of ex vivo islets in humans, it is expected that about 8,000-12,000 IdIs per patient kg may be required" to achieve the desired therapeutic effect.[4] The implant in USP '017 is designed to treat diabetes by creating a live

[1] Specification, page 8, line 2 through page 9, line 11.
[2] Final OA, mailed 11/03/05, page 2, paragraph 3.
[3] *Id.*, at page 5, paragraph 4.
[4] USP 6,703,017, col. 14, lines 7-9.

"insulin pump" in the body. Example 12 of USP '017 describes implanting 5,000 islets per NOD mouse, which is equivalent to 200,000 islet/kg of body weight. This treatment resulted in normoglycemia in these animals[5]. Thus, the '017 patent teaches the administration of only one fully-therapeutic dose of insulin-producing cells. It does not teach or suggest first implanting a tolerizing dose of insulin-producing cells prior to implanting the fully-therapeutic dose, wherein the tolerizing dose is at least one order of magnitude less than the therapeutic dose.

US Patent 5,425,764 describes a method of using an implantable bioartificial pancreas device containing insulin-secreting islets, to supply an exogenous source of insulin to treat the symptoms of diabetes[6]. Accordingly, the '764 patent also requires implantation of a fully therapeutic dose of insulin-secreting cells, i.e. the dose necessary to achieve normoglycemia. As such, the '764 Patent does not describe or suggest implanting a tolerizing dose of insulin-producing cells prior to implanting of a fully therapeutic non-encapsulated dose, where the tolerizing dose is at least one order of magnitude less than the therapeutic dose.

Similarly, US Patent 5,629,194 describes a method of implanting embryonic porcine pancreatic non-insulin-secreting cells capable of proliferating *in vivo* and then secreting insulin after transplantation. The dose sufficient for the treatment of insufficient insulin activity is 100,000-500,000 aggregates, each containing 300-500 cells per human patient[7]. This is a fully therapeutic dose. Thus, like the other two primary references, the '194 patent does not describe or suggest implanting a tolerizing dose of insulin-producing cells prior to implanting of fully therapeutic non-encapsulated dose, where the tolerizing dose is at least one order of magnitude less than the therapeutic dose.

The Examiner relied on a reference of Posselt et al. to show the administration of a tolerizing dose. In the Office Action mailed January 26, 2005, the Examiner cited Posselt et al., *Diabetes* 1992, **41**:771-775[8] (Posselt 1992). However, during the Examiner's interview conducted March 21, 2005, it was agreed that the correct reference should have been Posselt et

[5] *Id.* col. 23, line 48 through col. 24, line 5.
[6] USP 5,425,764 col. 2, lines 53-55; col. 6, lines 8-14; and claims 1-31.
[7] USP 5,629,194, col. 13, lines 12-28.
[8] Office Action, mailed 01/26/2005, page 5, paragraph 10.

al., *Ann. Surg.* 1991 **214**:363-373[9] (Posselt 1991). Nevertheless, in the Office action mailed June 16, 2005[10], and in the Final Office Action, mailed November 3, 2005, the Examiner again cited the Posselt 1992, reference[11]. It is clear that this is the wrong reference because in setting forth the bases for the rejection, the Examiner referred to the page numbers from the Posselt 1991 reference. Therefore, Appellant herein discusses the correct reference of Posselt 1991.

In the Final Office Action, the Examiner stated that "Posselet (sic) et al., teach two step strategy: first administering a small dose of cell that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site." The Examiner further states that "[t]here is no indication or suggestion in Posselt et al. that only intrathymic transplantation should be performed." And further: "it is noted that the instant claims does (sic) not recited (sic) any specific place where a first tolerizing dose of insulin-secreting cells should be implanting (sic)."[12] All of these statements are clearly incorrect.

First, although Posselt 1991 does disclose a two-step process of administering insulin-producing cells, the initial tolerizing dose is not "one to two orders of magnitude less than [a] therapeutic dose" as recited in pending Claim 3. The only doses of insulin-producing cells described in Posselt 1991 are described on page 364 of the reference under the heading "*Islet Isolation and Transplantation.*" 1000 to 1500 islets were transplanted into the liver and renal subcapsule, and 600 to 800 islets were transplanted into each lobe of the thymus, for a total of 1200 to 1600 islets. In the rats that did not destroy these islets, "serum glucose levels in these animals returned to normal within 48 to 72 hours after islet transplantation."[13] Thus, the dose that was applied was a fully therapeutic dose, rather than the tolerizing dose one to two orders of magnitude less than a therapeutic dose recited in Claim 3.

Moreover, the Examiner disagreed with Appellant's characterization of the Posselt 1991 reference as teaching away from delivery of the initial dose of insulin-producing cells to any site

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[9] Amendment filed May 6, 2005 (Summary of Interview).
[10] Office Action, mailed 06/16/2005, page 2, paragraph 3.
[11] Final Office Action, mailed 11/03/05, page 2, paragraph 3.
[12] *Id.*, page 2, last paragraph through page 3, first paragraph.

other than the Thymus. However, as explained below, one of ordinary skill in the art reviewing the Posselt 1991 reference would clearly conclude that the initial dose must be given to the thymus.

Posselt 1991 describes implanting unencapsulated islets into various areas of the body, including liver, kidney, and thymus, of spontaneously diabetic BB rats[14]. The only implantation site that showed survival of the implanted cells was the thymus[15]. The islets injected into the liver were rejected almost immediately, while islets injected into the kidney capsule had variable survival, with only two surviving as long as 120 days.[16] In contrast, the intrathymic islet recipients were observed for a period close to the life span of the rat, without any recurrent diabetes. There is no indication of any kind in the Posselt 1991 reference that any site other than thymus can be used to induce immunological tolerance. Indeed, the authors state several times in this article that thymus is considered to be an immunologically privileged site.[17]

In experiments conducted on intrathymically injected animals, approximately 100 days after the initial intrathymus transplantation, the transplanted rats were challenged with extrathymic allogeneic islets, which remained intact even after removal of the thymus bearing the islet allografts[18]. However, in animals which were able to maintain functional subcapsular islets for more than 120 days, the vigor of the immune response to subsequent allografts was not diminished[19]. As the authors stated several times in this article, thymus is considered to be an immunologically privileged site and is subject to the usual biologic characteristics of such sites, in that prior sensitization of the host with skin allografts precludes prolonged survival of intrathymic islets[20]. The experiments, performed by Posselt et al. show just that, i.e. when allogeneic islets were transplanted into the thymus of recipients that had previously rejected

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[13] Posselt et al.1991 "Intrathymic islet transplantation in BB rats" Ann. Surg. **214**:363-373 at page 365 left column.
[14] *Id.* at page 363, left column.
[15] *Id.* page 364, right column through page 365, right column.
[16] *Id* page 365, left column.
[17] *Id.* page 372, right column.
[18] *Id.* page 365, right column through page 366, left column.
[19] *Id.*, page 367, left column.

donor strain skin grafts, the islets were destroyed in an accelerated manner, demonstrating that the intrathymic site is readily accessible to activated T cells[21], and that no tolerance can be achieved using this protocol. Furthermore, Posselt et al. goes on stating that the achieved tolerization to intrathymic allografts is due to their direct influence on maturing thymocytes, which are more susceptible to tolerance-inducing signals, and that such "inappropriate" presentation of antigen by nonlymphoid cells induce a state of anergy in T cells[22].

Therefore, Posselt et al. clearly do teach away from using the initial dose of insulin-producing cells anywhere but thymus, and it only shows success in the absence of prior sensitization to the implant. If a skilled artisan was still looking for a way to solve the problem of creating tolerance to the implant other than described in the USP'017, USP'764, and USP'194, the publication of Posselt 1991 would point the artisan only in one direction: intrathymic implantation of a tolerizing dose of insulin-producing cells, because this reference convincingly teaches away from using tolerizing dose of insulin-producing cells anywhere but thymus, and it does not teach encapsulating these cells. Therefore, contrary to the Examiner's assertion, one skilled in the art would have no reasonable expectation of success in using the invention of the presently recited claims involving subcapsular, subcutaneous, intraperitoneal or intraportal implantation, and would have no motivation to do so after reading Posselt 1991.

Furthermore, the Examiner's statement that "the instant claims does (sic) not recited (sic) any specific place where a first tolerizing dose of insulin-secreting cells should be implanting (sic)" is simply inaccurate. All of the claims recite that the "implanting step is subcapsular, subcutaneous, intraperitoneal or intraportal." Thus, these claims clearly exclude implantation into the thymus.

Furthermore, as Dr. Scharp states in his Declaration submitted on May 6, 2005, the BB rat used as a model in Posselt 1991 has multitude of immunologic disorders that makes it more of a model for immune deficiency than a model for diabetes. Therefore, the BB rat is no longer

[20] *Id.* page 372, right column.
[21] Id. page 367 left column, and page 368, right column.
[22] Id. page 373, both columns.

considered an acceptable model for studying human autoimmune diabetes[23]. This is also stated by Posselt 1991: "BB rats are known to be significantly immunodeficient"[24].

The Applicant was the first one to teach that implantation of insulin-producing cells in sites other than thymus produces tolerance to the implanted cells. The Declaration of Dr. Scharp, submitted on May 6, 2005, reiterates that point.[25] Contrary to the Examiner's statement, the instant method as claimed in the present Claims 1-14 specifies the non-thymus implantation sites for the tolerizing dose of encapsulated cells. This limitation is not suggested in any of the cited references. Therefore, the cited references fail to suggest the claimed method. Accordingly, even when combined, these references do not teach all the limitations of the claimed invention. As such, the cited references fail to support a *prima facie* case of obviousness.

Additionally, with regard to Claim 3, none of the references including Posselt 1991 disclose or suggest an initial administration of a dose of cells less than a fully therapeutic dose. Accordingly, Claim 3 is nonobvious for this additional reason.

Finally, the claims recite that the insulin-producing cells are "encapsulated in a biologically compatible permselective membrane." One of ordinary skill in the art would not expect to inject such encapsulated cells into the thymus as suggested by Posselt 1991 because of the small size of the thymus.[26] Thus, one of ordinary skill in the art would not expect to be able combine the recited encapsulated cells with the methods of the three primary references and Posselt 1991. Thus, because the necessary motivation, teaching or suggestion to combine the references is absent, there is no *prima facie* case of obviousness for this additional reason as well.

Therefore, Claims 1-4 and 6-14 are in compliance with 35 U.S.C. 103(a), and the rejection of Claims 1-4, 6-11, 13 and 14 as obvious is clearly improper.

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[23] Declaration of Dr. Scharp, page 3, paragraph 8.
[24] Posselt et al. 1991 "Intrathymic islet transplantation in BB rats" Ann. Surg. **214**:363-373 page 365, left column.
[25] Declaration of Dr. Scharp, page 3, paragraph 10 through page 4, paragraph 16.
[26] A typical adult human thymus has a volume of 12 ml.

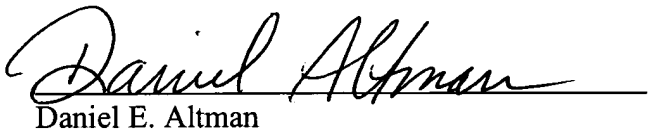
2. The Examiner has improperly rejected Claim 5 as being obvious. Non-obviousness of the independent Claim 1 in view of US Patent 6,703,017 or by US Patent 5,425,764, or US Patent 5,629,194 each in view of Posselt 1991 is asserted above. US Patent 5,529,914 discloses a method of encapsulating cells, but it fails to cure the deficiencies of US Patent 6,703,017, US Patent 5,425,764, US Patent 5,629,194, and Posselt et al. Therefore, Claim 5 is in compliance with 35 USC §103(a), and the rejection of Claim 5 as obvious is clearly improper.

Conclusion

In view of the arguments presented above, Appellants submit that the Specification as filed enables a person with an ordinary skill in the art on how to make and use the invention. Appellants further submit that Claims 2-9 are fully supported by the Specification as filed and do not constitute New Matter.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP.



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VIII. CLAIMS APPENDIX

1. **(Previously presented)** A method of treating diabetes in a mammal in need thereof, comprising the steps of:

implanting in said mammal a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane, wherein said implanting step is subcapsular, subcutaneous, intraperitoneal or intraportal; then

administering to said mammal a therapeutic dose of corresponding unencapsulated insulin-secreting cells.

2. **(Original)** The method of claim 1, wherein said mammal is a human, canine or feline.

3. **(Previously presented)** The method of claim 1, wherein said tolerizing dose is one to two orders of magnitude less than said therapeutic dose.

4. **(Original)** The method of claim 1, wherein said insulin-secreting cells are pancreatic islet cells.

5. **(Original)** The method of claim 1, wherein said membrane comprises polyethylene glycol.

6. **(Previously presented)** The method of claim 1, wherein said tolerizing and therapeutic doses comprise porcine cells.

7. **(Previously presented)** The method of claim 1, further comprising the step of administering one or more anti-inflammatory agents to said mammal prior to, at the same time as, or subsequent to administration of said therapeutic dose.

8. **(Original)** The method of claim 1, wherein said membrane has a molecular weight cutoff of about 150 kDa or less.

9. **(Original)** The method of claim 1, wherein said membrane has a pore size of less than about 0.4 μm.

10. **(Original)** The method of Claim 9, wherein said membrane has a pore size of less than about 0.2 μm.

11. **(Previously presented)** The method of Claim 1, wherein said therapeutic dose is between one and two orders of magnitude higher than said tolerizing dose.

12. **(Cancelled)**

13. **(Original)** The method of Claim 1, wherein said administering step is intraperitoneal, intraportal or subcutaneous.

14. **(Original)** The method of Claim 1, wherein said tolerizing dose is administered incrementally.

IX. EVIDENCE APPENDIX

1. Specification as filed;
2. Office Action, mailed January 26, 2005;
3. Office Action, mailed June 16, 2005;
4. Final Office Action, mailed November 3, 2005;
5. USP 6,703,017;
6. USP 5,425,764;
7. USP 5,629,194;
8. Posselt et al. 1991 "Intrathymic islet transplantation in BB rats" Ann. Surg. **214**:363-373;
9. Response to Office Action, filed May 6, 2005
9. Dr. D. Scharp Declaration under 37 CFR §1.132, filed May 6, 2005.

X. RELATED PROCEEDINGS APPENDIX

There are no decisions rendered by a court or the Board in any related proceedings identified above.

PTO/SB/08 Equivalent

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Multiple sheets used when necessary)

SHEET 1 OF 1

Application No.	10/823,263
Filing Date	April 13, 2004
First Named Inventor	Latta, Paul P.
Art Unit	1644
Examiner	Michail A. Belyavskyi
Attorney Docket No.	LATTA.002C4

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number *Number - Kind Code (if known)* Example: 1,234,567 B1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Foreign Patent Document *Country Code-Number-Kind Code* Example: JP 1234567 A1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear	T[1]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
MB	1	ATKINSON M.A. et al. (1999) "The NOD mouse model of type 1 diabetes: as good as it gets?" Nature Medicine 5:601-604.	

1776045:vr061705

Examiner Signature	Date Considered 10/21/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

T[1] - Place a check mark in this area when an English language Translation is attached.

PTO/SB/08 Equivalent

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Multiple sheets used when necessary)

SHEET 1 OF 1

Application No.	10/823,263
Filing Date	4-13-04
First Named Inventor	Latta, Paul P.
Art Unit	1644
Examiner	Belyavskyi, Michail A.
Attorney Docket No.	LATTA.002C4

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NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
MB	1	BINGLEY, P.J. et al. (1994) "Combined analysis of autoantibodies improves production of IDDM in islet cell antibody-positive relatives" Diabetes 43:1304(7).	
	2	BONIFACIO, E. et al. (1995) "Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity" Diabetologia 38:816-822.	
	3	CHRISTIE, M.R. et al. (1994) "Antibodies is islet 37k antigen, but not to glutamate decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity" Diabetes 43:1254(6).	
	4	LEE, H.C. et al. (1995) "Relationships among 64k autoantibodies, pancreatic beta-cell function, HLA-DR antigens and HLA-DQ genes in patients with insulin-dependent diabetes mellitus in Korea" Korean J. Intern Med. (Abstract only).	
	5	TUOMILEHTO, J. et al. (1994) "Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease" Lancet 343:1383-1385.	
	6	ZIMMET, P.Z. et al. (1994) "Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency" Diabetic Medicine 11:299-303.	
MB	7	ZIMMET, P.Z. et al. (1994) "Autoantibodies to glutamic acid decarboxylase and insulin in islet cell antibody positive presymptomatic type 1 diabetes mellitus: frequency and segregation by age and gender" Diabetic Medicine 11:866-871.	

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Examiner Signature	Date Considered 6/8/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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INDUCTION OF IMMUNOLOGICAL TOLERANCE

Related Applications

This application is a continuation of Application Serial No. 10/660,924 filed September 12, 2003 which is a continuation of Application Serial No. 09/226,742 filed January 7, 1999 now abandoned which was a continuation of Application Serial. No. 09/049,757 filed March 27, 1998 now abandoned, which was a continuation of Application Serial No. 08/736,413 filed on October 24, 1996 now abandoned, which claims the benefit of priority under 35 U.S.C. 119(e) of Provisional Application No. 60/005,877 filed October 26, 1995.

Field of the Invention

The present invention relates to the induction of immunological tolerance to foreign cells, tissues and organs. More specifically, the invention relates to implantation of a tolerizing dose of cells or tissues encapsulated in a membrane in a mammal to establish immunological tolerance thereto.

Background of the Invention

For some human diseases, including heart and liver failure, organ transplantation is the only alternative to certain death. While there were only 4,843 organ donors in the U.S. in 1993, there were 2,866 heart and 3,040 liver failure patients on the waiting list for these organs (*UNOS Update*, 10(2), 1994). Thus, because of timing and tissue matching problems, many patients die each year for lack of an available organ. For those lucky enough to receive an organ, the results are still less than ideal. The transplant procedure constitutes major surgery which is associated with attendant risks and is exceedingly expensive. After the surgery, the patient must be placed on a regimen of immunosuppressive drugs to keep the immune system from destroying the transplanted organ. As a consequence, the patient's entire immune system is suppressed for the rest of his life, significantly lowering his defenses against other serious disease threats such as infections, viruses or cancers.

For other diseases including kidney failure, pancreas failure and cystic fibrosis, transplantation has a lower mortality and morbidity rate than any alternative therapy. Even with its attendant problems of organ scarcity, surgical risk, high cost and permanent immunosuppression, for some of these cases it is still a more practical therapy than any alternative. The physician's choice in these cases is dependent on many variables including age, general health, severity of the condition, availability of organs and others factors. In 1994, there were 25,033 patients on the waiting list for human kidneys, 181 for pancreases and 1,250 for lungs (*UNOS Update*, 10:2, 1994).

For still other diseases, transplantation is known to be effective, although its attendant problems preclude its practical therapeutic use. This is true for many of the kidney, pancreas and lung patients described above. It is also true where whole pancreas transplantation can cure diabetes or liver transplantation can cure hemophilia but the risks outweigh the rewards.

Recently, for certain disease states; tissue transplants, as opposed to whole organ transplants, have been shown to be therapeutic in animals and even in man (Scharp et al., *Transplantation*, 51:76-85, 1991). Tissue transplantation requires full immunosuppression and carries the same risks and problems as already discussed for whole organ immunosuppression. The following treatments address the rejection of the transplanted tissue.

One implantation method involves pre-inoculation in the thymus with a small dose of cells, full temporary immunosuppression, then a full therapeutic dose at another site (Posselt et al., *Annals of Surgery*, 214:363-373, 1991). First, this has only been shown to work in rodents to date. No large animal or human test has been successful. Second, the human adult thymus is shrunken and may not be practical to treat with an adequate pre-dose. Third, the immunosuppression step, while temporary, does subject the patient to risks for that period of time. Fourth, it is not known whether a fully therapeutic dose will be tolerated, (i.e. not rejected) in humans. Fifth, this procedure may not protect against autoimmune destruction even if it does prevent rejection.

Another method of preventing rejection is irradiation of the recipient's bone marrow immune cells, implantation of bone marrow cells from the donor, then implantation of a full therapeutic dose of tissue or organ from the same donor (Illstad et al., *J. Exp. Med.*, 174:467-478, 1991). First, this has not been shown to work for tissue transplants in humans. Second, irradiation of immune cells, either partial or whole body, carries serious risks. Third, it is not known if the immune system will adequately protect from other threats. Fourth, it is not known if the method will protect from both rejection and autoimmune destruction in those disease states.

A further method of treatment to prevent rejection is by using monoclonal antibodies to suppress certain parts of the immune system (Andersson et al., *J.*

Autoimmun., 4:733-742, 1991). These tests have only been performed in rodents so it is not known if they would succeed in humans. Also, it is not known if the proper monoclonal antibody could be identified and created for each different disease state. In addition, the overall affect of these agents on the human immune system is not known.

Still another method of preventing rejection is encapsulation of the transplanted tissue in a semi-permeable membrane device which allows oxygen, nutrients and other small molecules to pass but prevents entry of large immune system cells (Lacy et al., *Science*, 254:1782-1784, 1991; Sullivan et al., *Science*, 252:718-721, 1991). There are several unresolved problems associated with this method. First, none of these devices has been shown to protect a therapeutic transplant in humans. To be suitable for human use, the material must be biocompatible; it must be sufficiently strong to last a long time when implanted; its porosity must be exactly correct to allow survival and function of the enclosed cells while keeping out cells and perhaps antibodies of the immune system; and finally, the device itself must be large enough to contain enough cells for a fully therapeutic implant and yet small enough to allow for some reasonable method of implantation which causes no damage to other internal organs.

To date, there has been very little effort to use transplantation as a potential prevention of disease due to all of the problems associated with transplantation as previously described. In addition, it is not yet known where transplantation can actually prevent a disease from occurring other than the obvious case of whole organ failures. Moreover, for many disease states, it is not known who will be afflicted. There is some evidence that interventional transplantation can have some preventive effect in rodents (Miller et al., *J. Neurol. Immunol.*, 46:73-82, 1993; van Vollenhoven et al., *Cell. Immunol.*, 115:146-155, 1988). Thus, a major role for preventive transplantation has not been investigated.

Summary of the Invention

One embodiment of the invention is a method of creating immunological tolerance to foreign cells, tissues or organs in a mammal, comprising the step of implanting in the mammal a tolerizing dose of foreign cells or tissue encapsulated in a biologically compatible permselective membrane. The method may additionally

comprise the step of administering to the mammal a curative dose of corresponding unencapsulated cells, tissue or organ. Advantageously, the mammal is a human, canine or feline. Preferably, the tolerizing cells are insulin-secreting cells; more preferably, they are pancreatic islet cells. According to one aspect of this embodiment, the membrane comprises polyethylene glycol. Preferably, the curative dose is between one and two orders of magnitude greater than the tolerizing dose. Advantageously, the tolerizing and curative doses are from the same species as the mammal. Alternatively, the tolerizing and curative doses are from a species different from the mammal. Preferably, the tolerizing and curative doses are porcine. The method may further comprise the step of administering one or more anti-inflammatory agents to the mammal prior to, at the same time as, or subsequent to administration of the curative dose. Preferably, the membrane has a molecular weight cutoff of about 150 kDa or less. Alternatively, the membrane has a pore size of about 0.4 μm or less. The membrane may also have a pore size of about 0.2 μm or less. Advantageously, when the tolerizing and curative doses are from a different species, the membrane has a molecular weight cutoff of about 150 kDa or less. Preferably, the tolerizing step is subcapsular, subcutaneous, intraperitoneal or intraportal and the curative step is intraperitoneal, intraportal or subcutaneous. The tolerizing dose may also be administered incrementally.

The present invention also provides a method of treating diabetes in a mammal in need thereof, comprising the steps of:

implanting in the mammal a tolerizing dose of foreign insulin-secreting cells encapsulated in a biologically compatible permselective membrane; then

administering to the mammal a curative dose of corresponding unencapsulated insulin-secreting cells.

Preferably, the mammal is a human, canine or feline. Advantageously, the tolerizing dose is one to two orders of magnitude less than the curative dose. In another aspect of this preferred embodiment, the membrane comprises polyethylene glycol. Advantageously, the insulin-secreting cells are pancreatic islet cells. Preferably, the mammal and the insulin-secreting cells are from the same species. Alternatively, the mammal and the insulin-secreting cells are from different species. Preferably, the

tolerizing and curative doses are porcine. The method may further comprise the step of administering one or more anti-inflammatory agents to the mammal prior to, at the same time as, or subsequent to administration of the curative dose. Advantageously, the membrane has a molecular weight cutoff of about 150 kDa or less. Alternatively, the membrane has a pore size of less than about 0.4 μm.

Brief Description of the Drawings

Figure 1 is plane view illustrating the key properties of the membrane enclosing the cells. The membrane may be configured into many different device designs.

Figure 2 is a plane view of one design of the invention, wherein two layers of the membrane are used in a flat sheet configuration where cells are "sandwiched" in between the two membranes and then the ends are sealed.

Figure 3 is a tubular view of one design of the invention, wherein the membrane is cast or rolled into a tubular configuration. The cells are loaded in the lumen and the ends are sealed.

Figure 4 is a spherical view of one design of the invention, wherein the membrane is cast in a spherical configuration and cells may be encased one in each device (microcapsule) or many in a device (macrocapsule).

Figure 5 is a graph showing blood glucose levels in mice implanted with a tolerizing dose of 100 encapsulated NIT insulinoma aggregates.

Figure 6 is a graph showing blood glucose levels in mice implanted with a tolerizing dose of 50 encapsulated NIT insulinoma aggregates.

Figure 7 is a graph showing blood glucose levels in non-tolerized control mice.

Detailed Description of the Preferred Embodiments

Goals of the Invention

The problems discussed in the foregoing Background of the Invention have previously not been solved for either micro or macroencapsulation of cells in humans. The present invention overcomes these problems associated with transplantation. Thus, one goal of the invention is to eliminate the critical problems of transplantation in cases where whole organ transplantation is the only alternative to certain death. These are cases of heart or liver failure. The major advantage of the invention process

for this application is that it eliminates the shortage of organs for the patients by making animal organs acceptable in humans. While there are only about 4,800 human organ donors in the U.S. each year, the supply of animal organs for transplant is not limited. The reason that animal organs are not presently used is that they are acutely rejected when transplanted into humans even with immunosuppression. Second, continuous immunosuppression is not required in the process of the invention, thus eliminating the risk of exposing the patient to other serious diseases while the immune system is suppressed. Third, the cost of organ transplantation is drastically reduced because of the unlimited supply of organs and because the continuous use of immunosuppressive drugs is not required.

A second goal of the invention is to make organ transplantation a safe, effective, practical therapy for those cases of disease where it is known now to be therapeutic but the risks associated with it prevent its widespread therapeutic use. Examples of these disease cases are kidney failure, pancreas failure and cystic fibrosis (lung failure). In these cases the advantages of the process of the invention eliminate the major obstacles. First, by making animal organs tolerated in humans the shortage of organs for these transplant needs is solved. Second, by eliminating the need for continuous immunosuppression, these patients are not exposed to other serious disease threats without a fully functioning immune system. Third, because of plentiful organs and no continuous immunosuppression, the cost of this transplant procedure would be greatly reduced.

A third goal of the invention is to make cell or tissue transplants, as opposed to whole organ transplants, a practical therapy in cases where cells or tissue alone can cure a disease state by providing a lacking or deficient protein, enzyme or peptide. Examples of these cases are insulin-secreting islet cells for Type I diabetes, Factor VIII-secreting hepatic cells for hemophilia, dopamine-secreting adrenal chromaffin cells for Parkinson's disease and collagen for arthritis. A significant advantage of the process of the invention for these cases is that animal tissue or genetically engineered tissue expressing an absent or deficient protein of interest can be used if human tissue is scarce. In addition, cell types other than the normal protein-secreting cells can be engineered to secrete the protein of interest. For example, myoblasts can be

engineered by standard methods to secrete insulin. The use of such cells is also within the scope of the present invention. Continuous immunosuppression is not needed to protect the transplanted tissue and the costs would be reduced. Thus, even if pre-inoculation into the thymus with immunosuppression or irradiation of bone marrow with immunosuppression or monoclonal antibodies could be identified and produced for many disease states or encapsulation of fully therapeutic doses of tissue in some membrane device can overcome many technical problems, the process of the invention is a safer and more practical therapy than any of these.

A fourth goal of the invention is the treatment of autoimmune diseases including diabetes, Alzheimer's, arthritis, multiple sclerosis, myasthenia gravis and systemic lupus erythematosus. In these diseases, the body's immune system attacks and destroys one's own tissue. By using the process of the invention, the immune system can be induced to accept grafted tissue or organs to replace those that have been destroyed without the autoimmune destruction of the newly transplanted graft. The advantage of this process is that organ rejection and autoimmune destruction are two completely different phenomena so that even with systems that may prevent rejection, in autoimmune diseases the grafts may still be destroyed by a different means. The process of the invention addresses both problems.

A fifth goal of the invention is to make transplantation a practical therapy to prevent certain diseases from ever occurring, as well as treating existing diseases as previously discussed. The advantage of the process that makes this possible is the immunomodulation effect which stops or prevents the immune system from destruction of self tissue. Thus, for all autoimmune disorders, the process can be used to intervene in the course of the disease at a critical point before the immune system is initiated into self-destruction of tissue that is necessary for normal body function.

As will be apparent from the ensuing detailed description of the invention, the present invention meets all of these goals. Additionally, the present invention also provides a number of advantages which would not have been readily apparent to one having ordinary skill in the art.

Overview

The present invention is a two step process. In the first step, a small number of cells or tissue is implanted into a mammal inside a device made of a biocompatible "permselective" membrane which protects the implanted cells from the mammal's immune system while at the same time allowing the cells to survive. A permselective membrane is one having a pore size selected so that it is small enough to prevent the entry of immunological factors such as cells or antibodies, yet large enough to allow the free passage of oxygen, nutrients and other molecules needed to sustain the transplanted cells. In addition, the membrane pores must allow the passage of antigens which are shed from the transplanted cells and prevent the entry of large immune system cells and antibodies. In a preferred embodiment, the mammal is a human. Alternatively, the mammal is a canine or feline.

One of ordinary skill in the art can readily determine the proper pore size for the permselective membrane for any particular application of the present invention. It is preferable to use the largest pore size possible to prevent the entry of the undesirable elements because the larger pores allow better diffusion of the desirable elements such as nutrients and oxygen across the membrane. Smaller pore sizes (e.g. those excluding molecules greater than 100,000 daltons) are not necessarily a problem for diffusion as has been shown in long-term survival of cells in a 50,000 dalton membrane *in vivo* implant (Lacy et al., *Science* 254:1782-1784, 1991).

Antigens shed from the transplanted cells pass through the permselective membrane into the body of the recipient where they are fully exposed to the immune system. The immune system will recognize these antigens as "foreign" and destroy them. This process will continue for some time with the immune system constantly destroying the shed antigens but not able to destroy the source which is the cells protected in the encapsulation device. In time, the immune system will begin to become tolerant of these antigens because they do no actual damage in the body and the constant source cannot be destroyed. At this time, the immune system is tolerant to that particular cell type from that particular donor.

Next, the second stage of the process is enacted. Now a fully therapeutic (curative) dose of cells, tissue or whole organ from the same donor as the tolerizing

dose is implanted in the recipient for cure of the disease. Since this implant, whether cells, tissue or organ, is from the same donor as the small dose, it is recognized by the immune system as "self" and a rejection response is not elicited. The immune system is fully tolerant to the new implant. In one embodiment, the tolerizing dose is given as a single (bolus) dose. Alternatively, the tolerizing dose may be administered incrementally over several weeks or months. In a preferred embodiment, the incremental tolerizing dose is the same as the bolus dose, only spread out in even increments. In another embodiment, the total incremental tolerizing dose is one to three times the bolus tolerizing dose. As for the bolus tolerizing dose, the incremental tolerizing dose is typically one to two orders of magnitude lower than the curative dose.

In addition to eliminating continuous immunosuppression, this process makes animal organs and cells available for human implants (xenografts). Presently, these organs or tissues are acutely rejected in humans because of the wide immunological barriers between the species. With the process of the invention, even animal tissue will be tolerated because tolerance is induced gradually over time. The availability of animal organs for human use will save many thousands of lives each year which are now lost due to the shortage of available human organs for transplantation. In addition, this process will allow transplant therapy for autoimmune diseases such as diabetes, arthritis, myasthenia gravis and multiple sclerosis. This is possible because as the immune system is tolerized to the new tissue by the initial small implant, the self-destructive autoimmune process is suppressed. So, for diseases requiring organs or cellular transplants, this process eliminates current shortages by making unlimited supplies of animal organs and cells available, eliminates the need for continuous immunosuppression, and protects the transplants from both rejection and autoimmune destruction. One particularly preferred source of xenograft cells or tissue for both the tolerization and curative steps is porcine cells or tissue.

Even with the tolerizing effect of the xenograft, because of the wide species differences, an initial inflammatory reaction may occur in response to the curative dose. Thus, in one embodiment of the invention, the xenograft recipient is administered one or more anti-inflammatory agents. The anti-inflammatory agent is

administered either systemically or locally at the implantation site. The agent may be administered prior to the implant, at the time of implantation or subsequent to the implant for a time necessary to overcome the initial inflammatory reaction. The agents may be over-the-counter preparations such as acetaminophen or ibuprofen, or a specific immunosuppressive agent such as Cyclosporine (Sandoz) or Imuran (azathioprine, Burroughs-Wellcome). The agent may also block the binding of a particular antigen such as CTLA4Ig (Bristol Myers Squibb). The amount of anti-inflammatory agent to be administered is typically between about 1 mg/kg and about 10 mg/kg. The extent of inflammation will determine whether the administration of such an agent(s) is necessary. The need for such agents is only temporary and not required for the ongoing survival and function of the transplant.

The process of the invention can also be used to prevent certain diseases, particularly autoimmune disorders. In these cases the process is as follows. First, patients at high risk for the disease or already in the very early phase of the disease are identified. At the critical time of the onset, the process is intervened with the small encapsulated tissue. For example, islets are used for Type I diabetes and collagen is used for arthritis. This implant of foreign tissue immediately diverts the attention of the immune system to the new foreign invader and it begins the process to destroy this new threat. Because of this diversion, the process of self-destruction of desirable tissue that was just beginning is suppressed, then abandoned, then forgotten. It is, in essence, "switched off" and the damage is prevented.

Implantation of cells to treat existing diseases

The first step of this method involves acquiring small amounts of cellular tissue for the initial tolerizing implant. The method in which tissue is obtained depends on the type of tissue needed, the source of the tissue, the donor, and the amount of tissue needed. These methods are generally well known by those skilled in the art of tissue digestion, separation, purification, culture, and the like. The following examples are only used to illustrate that these methods are readily available.

Islet cells for treatment of diabetes

Islets are small clusters of cells located in the pancreas of mammals. They are composed of alpha cells which make and secrete somatostatin, beta cells which make

insulin, delta cells which make glucagon and other cells which make other proteins. To isolate the islet cells which make up only 1-2% of the pancreas from the surrounding acinar tissue, the digestive enzyme collagenase is used. This process is described by Ricordi (*Diabetes* 37:413-410, 1988, hereby incorporated by reference). Once the islets are obtained, they are purified from acinar cells and can then be implanted fresh, cultured for extended periods, cryopreserved indefinitely or encapsulated.

For use in human treatments, primary islet cells are obtained from human cadaver donors or from suitable mammalian sources such as rat, cow, or pig. For use of animal tissue in humans, it is desirable to assure safety of the animal source by using specific pathogen-free (SPF) or gnotobiotic colonies or herds of animals. As an alternative to a primary cell source, an engineered cell line which is genetically altered to produce the proper regulated amounts of insulin, glucagon, somatostatin, etc. is also suitable for treatment of diabetes.

Adrenal chromaffin cells for Parkinson's disease, Alzheimer's and Huntington's disease

Adrenal chromaffin cells have multiple applications. They secrete the neurotransmitter dopamine for amelioration of Parkinson's disease, fibroblast growth factor, and can be engineered to secrete nerve growth factor which will counter degeneration and cell death in Alzheimer's and Huntington's disease. A collagenase digestion method of isolating adrenal chromaffin cells from the adrenal gland is described by Livett (*Physiol. Rev.* 64:1103-1161, 1984). Human or other mammalian sources can be appropriate sources of this tissue.

Moreover, mammalian cells can also be genetically engineered to secrete certain proteins or peptides whose absence or deficiency is the cause of various genetic diseases (i.e. adenosine deaminase deficiency). In addition, such cells can also be engineered to secrete various cytokines and growth factors for the treatment of viral infections (i.e., interferon-γ) and cancer (i.e., interleukin-2). Hormone deficiencies can also be treated by this method. Mammalian cells are transfected with an expression vector containing a gene encoding such a therapeutic protein or peptide. These expression vectors are constructed using standard methods well known to one of

ordinary skill in the art. A tolerizing dose of these cells is encapsulated as described herein and implanted into a mammal. Two to three weeks later, a curative dose of the same cells is implanted into the mammal. The cells are no longer recognized as foreign, are not destroyed by the host immune system and continue to secrete the desired therapeutic protein.

Other conditions treatable by encapsulated cells producing peptides, proteins or other therapeutic agents include hypoparathyroidism (thyroid hormone), hyperadrenocorticalism (adrenocorticotrophic factor), dwarfism (growth hormone), Gaucher's disease (glucocerebrosidase), Tay-Sachs (hexosaminidase A) and cystic fibrosis (cystic fibrosis transmembrane regulator). In addition, cells expressing stimulatory or inhibitory cytokines can be encapsulated, resulting in stimulation or inhibition, respectively, of a particular cell type. For example, erythropoietin stimulates red blood cell production, interleukin-2 stimulates the proliferation of tumor-infiltrating lymphocytes and interferons inhibit certain types of tumor cells. Other conditions contemplated for treatment using the method of the present invention include amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's Chorea, epilepsy, hepatitis, anxiety, stress, pain, addiction, obesity, menopause, endometriosis, osteoporosis, hypercholesterolemia, hypertension and allergies.

Other cell sources and methods for other diseases

Other cell/tissue sources and methods include collagen recovery from chicken for prevention and treatment of arthritis, Schwann cells from myelin tissue for prevention and treatment of neural degeneration and Factor VIII from liver hepatocytes for treatment and prevention of hemophilia.

The amount of cells or tissue necessary for the initial tolerizing implant will vary depending on the disease, site, source, whether the tissue is primary or immortalized and other factors. Generally, the tolerizing dose is one or two orders of magnitude less than a full dose implant. For example, in diabetes it usually takes between about 10,000 - 20,000 islets/kg of body weight to provide adequate insulin production for normoglycemia. Accordingly, the initial implant dose for tolerization is about 100 - 2,000 islets/kilogram of body weight. Although the size of these doses are not known for all disease states, they can be optimized using routine dose/response

experiments well known to one of ordinary skill in the art. In general, between about 100 cells/kg body weight and about 5,000 cells/kg body weight are suitable for tolerization. The corresponding curative doses are between about one and two orders of magnitude higher than these numbers.

Preparation of encapsulation device, loading of cells and implantation

The membrane for the device is chosen for the application needed based on its biocompatability, permeability, strength, durability, ability to be manipulated and other important considerations. A number of materials have already been shown to be acceptable for implants in mammals. Examples of some of these materials are PAN/PVC acrylic co-polymers, hydrogels such as alginate or agarose, mixed esters cellulose, polytetrafluoroethylene (PTFE)/polypropylene (Lum et al., *Diabetes* 40:1511-1516, 1991; Aebischer et al., *Exp. Neurol.*, 111:269-275, 1991; Liu et al., *Hum. Gene Ther.* 4:291-301, 1993; Hill et al., *Cell Transplantation* 1:168, 1992, all hereby incorporated by reference) and polyethylene glycol (PEG) conformal coating configurations (U.S. Patent No. 5,529,914, hereby incorporated by reference).

A critical factor is the pore size that can be produced in the material chosen. For example, PEG macromers can vary in molecular weight from 0.2 - 100 kDa. The degree of polymerization, and the size of the starting macromers, directly affect the porosity of the resulting membrane. Thus, the size of the macromers are selected according to the permeability needs of the membrane. It is believed that for xenograft transplants (animal to human), antibodies of the immune system and complement are involved in rejection (Bachet al. *Transplantation* Overview 6(6):937-947, 1991). In this case, a pore size (molecular weight cutoff) of 150 kDa or smaller is desired to prevent the passage of the smallest immune antibody (IgG) through the pores of the membrane capsule. Thus, the application and its conditions will determine the choice of membrane material from many available alternatives. Likewise, the configurations of the device will be determined by the application. For purposes of encapsulating cells and tissue in a manner which prevents the passage of antibodies across the membrane but allows passage of nutrients essential for cellular metabolism, the preferred starting macromer size is in the range of about 3 kDa to 10

kDa, with the most preferred being about 4 kDa. Smaller macromolecules result in polymer membranes of a higher density with smaller pores.

It is also believed that for allografts (human to human), only entry of immune system cells must be blocked to prevent rejection of transplanted tissue or organs (Auchincloss, Jr., *Transplantation* Overview 46(1):1-20, 1988. In addition, it is also desirable to exclude other cells, the smallest of which are red blood cells which have a size of about 7 μm. Accordingly, a membrane having a molecular weight cutoff of about 150 kDa is also suitable for encapsulation of allograft cells or tissue because such membranes will prevent entry of such cells. In an alternative embodiment, the pore size for allografts is about 0.4 μm or less to prevent the entry of immune and non-immune cells into the device. Cells can also extend processes ("arms") which can enter openings having a size of about 0.2 μm. Thus, in another preferred embodiment, the pore size is about 0.2 μm or less. In a most preferred embodiment, the pore size is as small as possible to exclude entry of detrimental components, but allows cell survival by permitting vital molecules such as nutrients, proteins and oxygen to freely pass through the permselective membrane. A desired pore size may be obtained by adjusting the crosslink density and length of PEG segments by one of ordinary skill in the art without undue experimentation.

If retrieval of the initial implant is unnecessary or undesirable, then a suitable configuration may be microcapsules where only a few or even single cells are each enclosed in separate membranes. Because of the small volume in this case, the microcapsules may simply be injected into one of many sites for the implant. If it is desirable to retrieve or reload the device or larger numbers of cells are necessary, a "macrocapsule" may be constructed wherein many cells are enclosed together inside one membrane. In this case, it has been shown that the environment inside the macrocapsule may need special conditions to allow the cells to survive. For example, an alginate matrix has been used to immobilize islet cells and prevent their aggregation and subsequent central necrosis (Lacy et al., *Science*, 254:1782-1784, 1991).

For other cell types a different environment may be needed. The macrocapsule may be of any shape that is practical. Examples of shapes commonly used by those

skilled in the art are: 1) flat sheet "sandwiches" where two layers of the membrane are top and bottom on the cells and the ends are sealed by heat welding, gluing, or other known means (Fig. 2). This method provides a large surface area for membrane exposure to the host systems and generally short diffusion distances which helps transport substances across the membrane; 2) A tubular membrane formed by co-extrusion or rolling a flat sheet into a tube and sealing the ends (Fig. 3).

The cells can be placed inside the lumen at the same time the membrane is formed if co-extrusion is employed. If the tube is made first, the cells are loaded by syringe or other means and the ends are sealed by heat welding, gluing or other known means. As previously discussed, various matrices may be employed as needed by the enclosed cells. The tubes can be any suitable length and may be joined at the ends (potted) or woven if multiple tubes are used; 3) a spherical shape (Fig. 4) which has a large surface area compared to its volume and is efficient in some applications.

These are only illustrative examples of how membranes may be configured into devices to hold cells. One of ordinary skill in the art will appreciate that many more configurations are possible, thus providing great flexibility for many conceivable applications.

The loaded devices are then implanted into patients in need of therapy. The method of implantation, site and duration are dependent on the disease being treated. For example, in diabetes it is desirable to have the shed antigens processed by the liver. Therefore, implantation in the peritoneum where the portal circulation would carry the antigens directly to the liver (intraportal) is a preferred site. Alternatively, if the dose is a small enough volume (i.e. 10 µl or less), direct injection into the portal vein is preferred. Other implantation sites include under the kidney capsule and subcutaneous implantation.

For Parkinson's disease, the cells should be processed first in the brain. Thus implanting into the interstitial region of the brain is a preferred site. For each site, the method of implantation may be different. For example, intraperitoneal placement of a device for diabetes may be performed by a minimally invasive laparoscopic procedure. To place a device in the brain, the neurosurgeon commonly uses stereotaxic instruments to ensure exact placement. For a subcutaneous implant, a

small incision to allow a trocar to be inserted may be used. For each preferred site, those skilled in the art will recognize the most efficient method of implantation.

Once implanted, the cells are left in place for a period of time during which tolerization will occur. This time period will vary depending on the disease treated, whether an allograft or a xenograft transplant is being used, site of the implant, and other factors. Generally, tolerization requires from a few weeks to a few months. During this time, the transplanted cells constantly shed antigens from their surface. These antigens comprise a variety of small molecules which are constantly being replaced by living cells The antigens can pass freely out of the pores of the membrane and into the recipient at the locations of the implant and eventually into the circulatory system. The immune system immediately recognizes these antigens as "foreign" and initiate its mechanisms to protect the recipient from the intruder. These mechanisms are complex and not completely understood. Generally, it is believed that if the foreign matter is from a closely related species (allogeneic), cells of the immune system play the primary role in the immunological response. These cells include T-cells, macrophages, neutrophils, and natural killer cells which seek out the source of the invasion and destroy the foreign matter. If the foreign matter is a transplanted xenogeneic organ, preformed antibodies cause hyperacute (within minutes) reaction and rejection of the organ. If the foreign matter is xenogeneic cells or tissue, the antigen may not be presented and the preformed antibodies may not be the primary mechanism of rejection. Instead, macrophages stimulate killer T-lymphocytes and later (8-10 days) antibody stimulation causes final rejection of cells or tissue.

In the present invention, however, neither system can destroy the cells of the implant when the pore size of the membrane is properly selected for the application. For example, if allografts are destroyed by immune cells, then the membrane pores must only prevent entry of these cells and thus may be about 0.4 μm or smaller. Likewise, if it is necessary to prevent antibodies from reaching the cells, the pores must be smaller than the smallest of the human antibodies, IgG, which is 150 kDa. Of course, a pore size having a molecular weight cutoff of about 150 kDa or less is suitable for tolerization in both allografts and xenografts.

The use of a permselective membrane prevents the immune system from destroying cells encapsulated therein, even though the immune system recognizes the implant tissue as foreign and mounts a classical response. The immune response cannot destroy the cells because they are protected within the membrane device. Because the immune system cannot destroy the cells even over time, the system will come to tolerate the implant and cease trying to destroy it. While the mechanism for this tolerization is not known, it is analogous to desensitizing patients to allergic immune reactions (i.e. antibiotics or bee stings). In fact, an alternative method to the single tolerizing implant is the addition of more cells with more implants over time if necessary. At this point, the immune system basically recognizes this cellular material as "self" and no longer mounts an immune response against it.

Implantation of full curative dose

When the patient has been tolerized to the cells of the implant, a full curative curative size dose of the tissue or whole organ is administered as described in the following examples.

Whole organ transplants - allografts

In one embodiment, the method is used for a human allograft. In this embodiment, the tissue for the initial implant is taken from a living related kidney donor by biopsy or similar method and a tolerizing dose is implanted into the patient. When the patient is tolerized, the whole kidney is taken from the donor and transplanted into the recipient. The graft is accepted with no continuous immunosuppression being necessary.

Whole organ transplants - xenografts

For most embodiments, it is preferable to use animal organs for human transplants. In these embodiments, the procedure is as follows: suitable animal donors are identified. Sources of these donors may be genetically identical (inbred). Tolerizing cells are taken from any animal in the colony. Later, the whole organ is taken from any other animal in the colony. It is preferable that these sources are free of all contaminants of risk to humans so they would preferably be specific pathogens free (SPF) or gnotobiotic (totally isolated in sterile conditions) colonies or herds. Heart, lungs, livers, kidneys, pancreases and other organs may be used in this

embodiment, thus eliminating the critical shortage of these organs from the limited number of available human organ donors.

Cellular transplants - allografts

In this embodiment, the method is used for human to human cellular transplants. A full size therapeutic dose is obtained from the cadaver donor source as previously described. For example, islet cells are obtained from the pancreas of a human donor. The small amount needed for the tolerizing implant is taken from the preparation, encapsulated and implanted as previously described. The remainder of the cells are cryopreserved by known methods (Kneteman et al, *Transplant. Proc.* 18:182-185, 1986) and are held until tolerization is completed. The full preparation is then thawed and ready for implantation. If, in this embodiment, it is necessary to acquire cells from more than one donor to have enough for a curative implant, then the cells for the initial implant are taken from multiple donors and mixed for the implant. The recipient is therefore tolerized to all of the cells from the multiple donors.

Cellular transplants - xenografts

As with whole organs, the present method allows the use of cellular transplants from animals as well. Cells for the initial implant are taken from genetically identical animals or multiple pooled animals as previously described. When the individual is ready for the full transplant, cells may be taken from any other member of the genetically identical colony or from multiple pooled animals if necessary for sufficient curative quantities.

The implantation procedure for the fully curative dose of cells, whether allograft or xenograft, is dependent on the disease, the quantity of cells, the site, and other factors. For example, for diabetes, a preferred procedure for the implantation of islet cells in humans is to inject the cells through the portal vein so that they become lodged in lobes of the liver. This procedure is done under local anesthesia and is minimally invasive to the patient. For treatment of neural disorders, cells can be implanted into any selected area of the brain by well known stereotaxic surgical procedures. Those skilled in the art will know preferred methods for cellular implantation for each embodiment.

Implants for Prevention of Diseases

Identification of patient populations is dependent on the ability to diagnose patients at high risk of developing certain diseases or those in early stages of the disease. Rapid progress has been made in this area of medicine primarily due to major advances in understanding and mapping the human genome. In addition, DNA amplification methods, notably the polymerase chain reaction (PCR), can be used to diagnose certain genetic disorders. Other research areas for predicting diseases are advancing as well.

In diabetes, the use of immune marker autoantibodies to establish preclinical diabetes has been well studied (Palmer, *Diabetes Rev.* 1(1):104-116, 1993). When these patients are identified, the physician determines at what point in the course of the disease it would be most advantageous to intervene.

Individuals determined to be at risk for development of a particular disease are implanted with the appropriate cell type as described above. Methods for acquiring small amount of cellular tissue for the initial tolerizing implant, tissue types, the amount of tissue necessary for implantation, preparation of the encapsulation device, loading cells into the device, implanting the device into a patient, membrane parameters, device configuration, implantation methods, curative dose administration, etc. are the same as discussed hereinabove for disease treatment.

Treatment of Diseases Arising from Lack of a Hormone

A study was performed using an insulin-producing mouse tumor cell line encapsulated in a permselective membrane coating as described in the following example.

Example 1

Implantation of mouse insulinoma cells

The NIT insulin-producing mouse tumor cell line was encapsulated with PEG conformal coatings of a single configuration, 11% PEG 4,000 kDa molecular weight (See U.S. Patent No. 5,529,914), which corresponds to a molecular weight cutoff of between about 10 kDa and about 70 kDa. The encapsulated cells were implanted beneath the kidney capsule at two different doses into C57B6 mice of a different allograft haplotype in which diabetes had been induced by intravenous injection (tail

vein) of 167 mg/kg body weight of streptozotocin (Upjohn, Kalamazoo, MI). Induction of diabetes by streptozotocin injection is a well known procedure which destroys pancreatic insulin-producing β cells.

Tolerizing doses of encapsulated insulinoma cells were 50 or 100 cell aggregates, each containing about 1,000 cells. Encapsulated cells were implanted beneath the kidney capsule using standard surgical procedures. Curative implants of unencapsulated insulinoma cells (2,000 - 3,000 insulinoma cell aggregates, each containing about 1,000 cells) were administered by free intraperitoneal injection 15 or 20 days after the tolerizing dose to determine whether a sufficient quantity of cells survived. Control animals were given only the curative dose of insulinoma cells. Blood glucose levels were monitored and are shown for the 100 encapsulated NIT aggregate tolerizing dose, 50 encapsulated NIT aggregate tolerizing dose and non-tolerized controls (Figures 5, 6 and 7, respectively).

The severity of streptozotocin-induced diabetes in these mice caused several of the animals to die during the periods of observation and during procedures done as part of the study. Table 1 indicates the number of animals involved in the study and their outcomes. The degree of diabetes is very high, with values over 500 mg/dl (shown as 500) for all streptozotocin-induced animals in the study. Many of these severely diabetic animals died of their diabetes during the study or following a procedure as noted. As shown in Figure 5, of the first group of 8 diabetic mice receiving 100 encapsulated aggregates, only four survived for the challenge 20 days later with the unencapsulated aggregates. Two of these died overnight following the IP injection. The remaining two recipients both had a sudden and marked reduction in their glucose values between 5 and 9 days, with glucose values reaching levels of 40 mg/dl and below (BM5 and BM11, Figure 5 and Table 1). If the insulin-secreting insulinoma cells induce immunological tolerance, the curative implant will be recognized as "self" and will not be destroyed by the recipient's immune system. Because the NIT cells are tumor cells which double every 2-3 days *in vitro*, their survival would be expected to result in recipient hypoglycemia due to the increasing insulin-producing cell mass that would occur from living and growing tumor cells.

In the second group of three recipients of 50 encapsulated aggregates for 15 days, two died of their diabetes prior to challenge with unencapsulated NIT cells. The one animal that received the challenge of unencapsulated NIT cells (BM16) has not exhibited any reduction in blood glucose values for the same time of observation (Figure 6). None of the control animals only challenged with unencapsulated NIT cells exhibited any reduction in blood glucose values (Figure 7).

The results indicate that encapsulated NIT cells given as a small mass prior to a large, unencapsulated curative cell implant permits the second curative dose to survive, reducing blood glucose values in a pattern suggestive of NIT tumor cell growth. A smaller dose of encapsulated NIT cells did not give this result. Control animals that only received unencapsulated NIT cells in a curative dose exhibited no reduction in blood glucose. These results indicate that the preliminary encapsulated implant tolerized the host to the following unencapsulated curative dose. When such a preliminary encapsulated implant was not done, the curative unencapsulated implants had no effect on blood glucose and were presumably destroyed by the host.

Table 1

Animal #	Toler. Encap. Implant	# Encap. Cell Agg. Tol. Dose	Delay to Cure Implant	Unencap. Cell Implant	# Unenc. Cell Agg. Cure	Effect on Blood Glucose
BM1	yes	100	20 days	yes	2348	none-died*
BM3	yes	100	20 days	no-died	-	n/a
BM4	yes	100	19 days	no-died	-	n/a
BM5	yes	100	20 days	yes	2348	down to 40
BM6	yes	100	20 days	no-died	-	n/a
BM7	yes	100	20 days	yes	2348	none-died*
BM10	yes	100	20 days	no-died	-	n/a
BM11	yes	100	19 days	yes	2348	down to 40
BM14	yes	50	15 days	no-died	-	n/a
BM15	yes	50	15 days	no-died	-	n/a
BM16	yes	50	15 days	yes	2348	none-500

Animal #	Toler. Encap. Implant	# Encap. Cell Agg. Tol. Dose	Delay to Cure Implant	Unencap. Cell Implant	# Unenc. Cell Agg. Cure	Effect on Blood Glucose
BM29	no	0	-	yes	2352	none-500
BM31	no	0	-	yes	2352	none-500
BM32	no	0	-	yes	2352	none-500
BM34	no	0	-	yes	2352	none-500
BM35	no	0	-	yes	2352	none-died*
BM36	no	0	-	yes	2352	none-500
BM40	no	0	-	yes	2352	none-500
BM41	no	0	-	yes	2352	none-died*
BM42	no	0	-	yes	2352	none-500
BM43	no	0	-	yes	2352	none-died*
BM44	no	0	-	yes	2352	none-500
BM45	no	0	-	yes	2352	none-500

* Died during course of experiment-no effect on blood glucose

Example 2

Use of encapsulated islets for induction of allograft tolerance in rats

Rat pancreatic islet cells are isolated by a standard collagenase digestion method (Ricordi, *Diabetes* 37:413-410, 1988) and cultured for three days prior to PEG encapsulation. Donor islets are derived from the Wistar Furth (WF) strain having MHC haplotype RT1-U. Recipients are of the Lewis strain having MHC haplotype RT1-1. Transplants across this strain combination are normally rejected within three weeks. Islet transplant mass is dosed on the basis of a standard 150 μm diameter rat islet; an Islet Equivalent (Ieq). Islets are quantified and tested for sterility and mycoplasma prior to encapsulation and implantation.

Islet cells are conformally coated with 11% PEG 4,000 kDa molecular weight by the method described in U.S. patent No. 5,529,914. As a negative control, acellular cross-linked dextran beads are encapsulated in a similar manner. Diabetes is induced in fasted Lewis rats by intravenous injection of streptozotocin (65 mg/kg) one week prior to implantation of the tolerizing dose and monitored during that week for blood glucose levels and weight changes. Rats are considered diabetic once their

blood glucose level exceeds 350 mg/dl. Rats having a minimal weight loss and blood glucose levels of 300-350 mg/dl are used for the study.

Diabetic rats are implanted by trochar with a subcutaneous 30 day time release depot insulin (Linplant, Lishin, Ontario, Canada) to reduce the chances of ketosis/acidosis and to stabilize their diabetes. Animals remain hyperglycemic at this Linplant dose (2 units of bovine insulin in 24 hours - lasts 30 days).

Diabetic MHC disparate Lewis rats are surgically implanted once with encapsulated donor WF islets at the renal subcapsular site after anesthetization. The dose of implanted cells varies as outlined in Table 2.

Table 2

Group	N	Dose	Rationale
1	12	1200 encap islets	high dose sensitization/tolerization
2	12	600 encap islets	low dose tolerization
3	12	300 encap islets	very low dose tolerization
4	12	1200 encap acellular beads	control for polymer

As a control, a set of recipients (Group 4) is implanted with encapsulated acellular beads to control for possible polymer effects in tolerization. All implanted animals are maintained for intervals as shown in Table 3 prior to the second transplantation. At the time of implantation, serum samples from each animal are drawn and retained for future immunological analysis.

Table 3

Group	N	Dose	Implant Interval (days)	Rationale
1a	4	1200 encap islets	30	high dose sensitize/tolerize-short interval
1b	4	1200 encap islets	60	high dose sensitize/tolerize-intermediate interval
1c	4	1200 encap islets	90	high dose sensitize/tolerize-long interval
2a	4	600 encap islets	30	low dose tolerization-short interval
2b	4	600 encap islets	60	low dose tolerization-intermediate interval
2c	4	600 encap islets	90	low dose tolerization-long interval
3a	4	300 encap islets	30	very low dose tolerization-short interval
3b	4	300 encap islets	60	very low dose tolerization-intermediate interval
3c	4	300 encap islets	90	very low dose tolerization-long interval

4a	4	1200 encap acell-beads	30	polymer control-short interval
4b	4	1200 encap acell-beads	60	polymer control-intermediate interval
4c	4	1200 encap acell-beads	90	polymer control-long interval

During the indicated period, animals are monitored for weight changes and blood glucose levels. One week before the second transplant, one animal in each of Groups 1a-1c, 2a-2c, 3a-3c and 4a-4c is sacrificed and the implant site analyzed by histological methods for determining viability of the tolerizing cells.

Lewis rats remaining in Groups 1-4 receive a second transplant (curative dose) of WF islets which are unencapsulated. Transplant sites in each animal are intraportal (IP) at a dose of 6,000 Ieq and at one kidney with a dose of 100 Ieq (See Table 4). 6,000 Ieq implanted into the liver is known to be a curative dose in the rat diabetes model. The 100 Ieq kidney capsule implant is only for histology at the end of the experiment. At the time of the second implant, serum samples from each animal are drawn and retained for future immunological analysis. For the next three weeks, animals are monitored for blood glucose levels and weight changes. At the termination of the experiment, graft sites are processed for histology. At this time, serum samples from each animal are again drawn and retained for future immunological analysis.

Table 4

Group	N	Recipient haplotype	Dose # of islets	Implant Sites	Duration of Transplant
1a	3	RT1-1	6000	IP/kidney	3 weeks
1b	3	RT1-1	6000	IP/kidney	3 weeks
1c	3	RT1-1	6000	IP/kidney	3 weeks
2a	3	RT1-1	6000	IP/kidney	3 weeks
2b	3	RT1-1	6000	IP/kidney	3 weeks
2c	3	RT1-1	6000	IP/kidney	3 weeks
3a	3	RT1-1	6000	IP/kidney	3 weeks
3b	3	RT1-1	6000	IP/kidney	3 weeks
3c	3	RT1-1	6000	IP/kidney	3 weeks

4a	3	RT1-1	6000	IP/kidney	3 weeks
4b	3	RT1-1	6000	IP/kidney	3 weeks
4c	3	RT1-1	6000	IP/kidney	3 weeks

In Groups 1 and 4, no changes in the diabetic state are measured. In Group 4, rejection occurs in the expected two week time frame as measured by a transient normoglycemia followed by a return to the diabetic state. In Group I, a more rapid rejection of the implant due to sensitization of the recipients occurs. In the recipients previously exposed to tolerizing doses of encapsulated WF islets (Groups 2 and 3), islet cells survive and result in a continuous maintenance of normoglycemia.

Example 3

Use of encapsulated islets for induction of allograft tolerance in humans

Human islets are isolated from cadavers and 1,500 islets/kg body weight are PEG-encapsulated and implanted under the kidney capsule in a diabetic patient. After two months, a curative dose of 15,000 unencapsulated islets/kg body weight are injected intraportally. Insulin administration is continued during the course of the protocol up to administration of the curative dose. Blood glucose levels are constantly monitored and are within the normal range.

Example 4

Treatment of Parkinson's disease (xenograft)

Adrenal chromaffin cells are isolated from inbred baboon adrenal glands and 1,000 cells/kg body weight are encapsulated in an appropriate PEG conformal coating. The capsule is implanted into the interstitial brain region of a human by a neurosurgeon using stereotaxic instruments. After 1 month of tolerization, 10,000 unencapsulated cells/kg body weight are injected into the same brain region. Significant improvement in the condition is observed.

Example 5

Prevention of hemophilia

A male individual at risk of developing hemophilia, an x-linked disorder, by virtue of family history, is subjected to genetic screening to determine the presence or absence of the gene encoding Factor VIII, and to clotting time analysis. If the gene

is absent or clotting time is reduced, 2,500 liver cells/kg recipient body weight are isolated from a human donor and encapsulated in a PEG conformal coating. The encapsulated cells are implanted under the kidney capsule. One month later, 5,000 cryopreserved liver cells/kg recipient body weight (from the same donor) are injected intraportally. Clotting time is significantly improved.

Example 6

Liver transplant (xenograft)

An individual in need of a liver transplant is subcutaneously implanted with 1,000 PEG-encapsulated liver cells/kg body weight isolated from an inbred baboon. Two months later, the entire liver is transplanted into the individual. Signs of organ rejection and vital signs are monitored over several months. Rejection does not occur.

Example 7

Prevention of myasthenia gravis (xenograft)

Myasthenia gravis is an autoimmune disorder resulting from the presence of antibodies against the acetylcholine receptor on neurons. An individual having very early signs of the disease is implanted under the kidney capsule with a tolerizing dose of 2,500 PEG-encapsulated neural cells/kg recipient body weight expressing the acetylcholine receptor isolated from baboons. This results in tolerization to the acetylcholine receptor and prevention of the disorder.

It should be noted that the present invention is not limited to only those embodiments described in the Detailed Description. Any embodiment which retains the spirit of the present invention should be considered to be within its scope. However, the invention is only limited by the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating diabetes in a mammal in need thereof, comprising the steps of:

implanting in said mammal a tolerizing dose of insulin-secreting cells from the same species as said mammal encapsulated in a biologically compatible permselective membrane; then

administering to said mammal a curative dose of corresponding unencapsulated insulin-secreting cells.

2. The method of claim 1, wherein said mammal is a human, canine or feline.

3. The method of claim 1, wherein said tolerizing dose is one to two orders of magnitude less than said curative dose.

4. The method of claim 1, wherein said insulin-secreting cells are pancreatic islet cells.

5. The method of claim 1, wherein said membrane comprises polyethylene glycol.

6. The method of claim 1, wherein said tolerizing and curative doses are porcine.

7. The method of claim 1, further comprising the step of administering one or more anti-inflammatory agents to said mammal prior to, at the same time as, or subsequent to administration of said curative dose.

8. The method of claim 1, wherein said membrane has a molecular weight cutoff of about 150 kDa or less.

9. The method of claim 1, wherein said membrane has a pore size of less than about 0.4 μm.

10. The method of Claim 9, wherein said membrane has a pore size of less than about 0.2 μm.

11. The method of Claim 1, wherein said curative dose is between one and two orders of magniture higher that said tolerizing dose.

12. The method of Claim 1, wherein said implanting step is subcapsular, subcutaneous, intraperitoneal or intraportal.

13. The method of Claim 1, wherein said administering step is intraperitoneal, intraportal or subcutaneous.

14. The method of Claim 1, wherein said tolerizing dose is administered incrementally.

INDUCTION OF IMMUNOLOGICAL TOLERANCE

Abstract of the Invention

A method of creating tolerance to transplanted cells, tissue, or organs without the need for continuous immunosuppression. A tolerizing dose of a cell or tissue within a membrane structure is implanted into a patient. Once the patient becomes tolerant to the cell or tissue, a tissue or organ is implanted which will no longer be recognized as foreign matter. The method makes animal organs practical for human use, prevents autoimmune destruction as well as immune rejection. It has applications in treatment and prevention of many mammalian diseases.

NSB-3787
102496

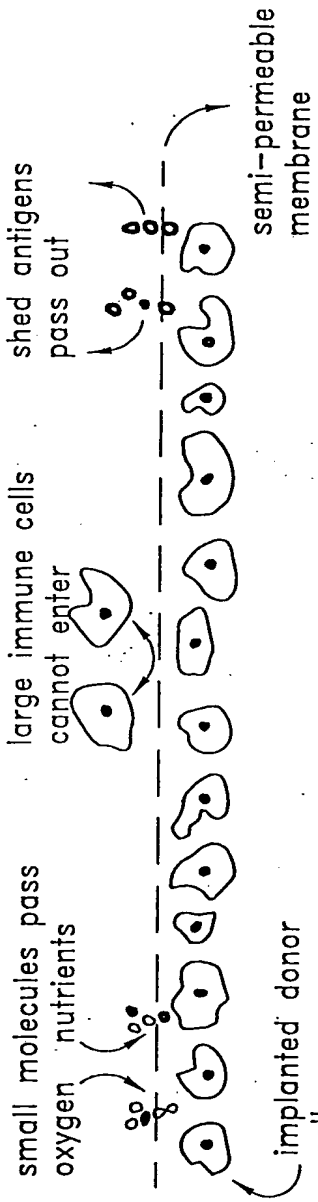
small molecules pass
oxygen nutrients
large immune cells
cannot enter
shed antigens
pass out
semi-permeable
membrane
implanted donor
cells

FIG. 1

FIG. 4

FIG. 3

FIG. 2

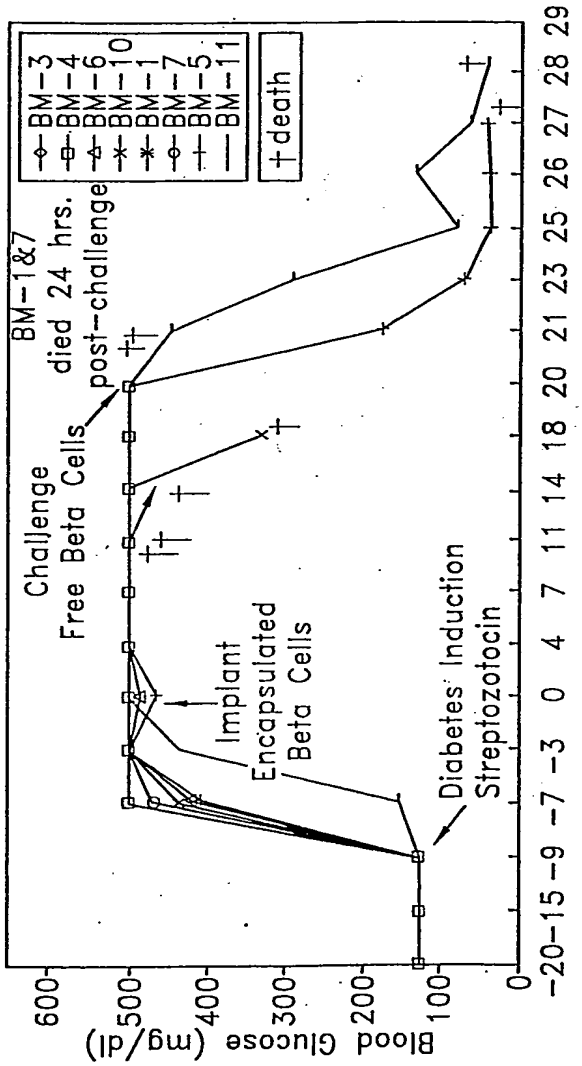
Blood Glucose (mg/dl)
600
500
400
300
200
100
0
-20 -15 -9 -7 -3 0 4 7 11 14 18 20 21 23 25 26 27 28 29
Days Post Capsule Implantation
BM-3
BM-4
BM-6
BM-10
BM-1
BM-7
BM-5
BM-11
†death
Challenge Free Beta Cells
BM-1&7 died 24 hrs. post-challenge
Implant Encapsulated Beta Cells
Diabetes Induction Streptozotocin

FIG. 5

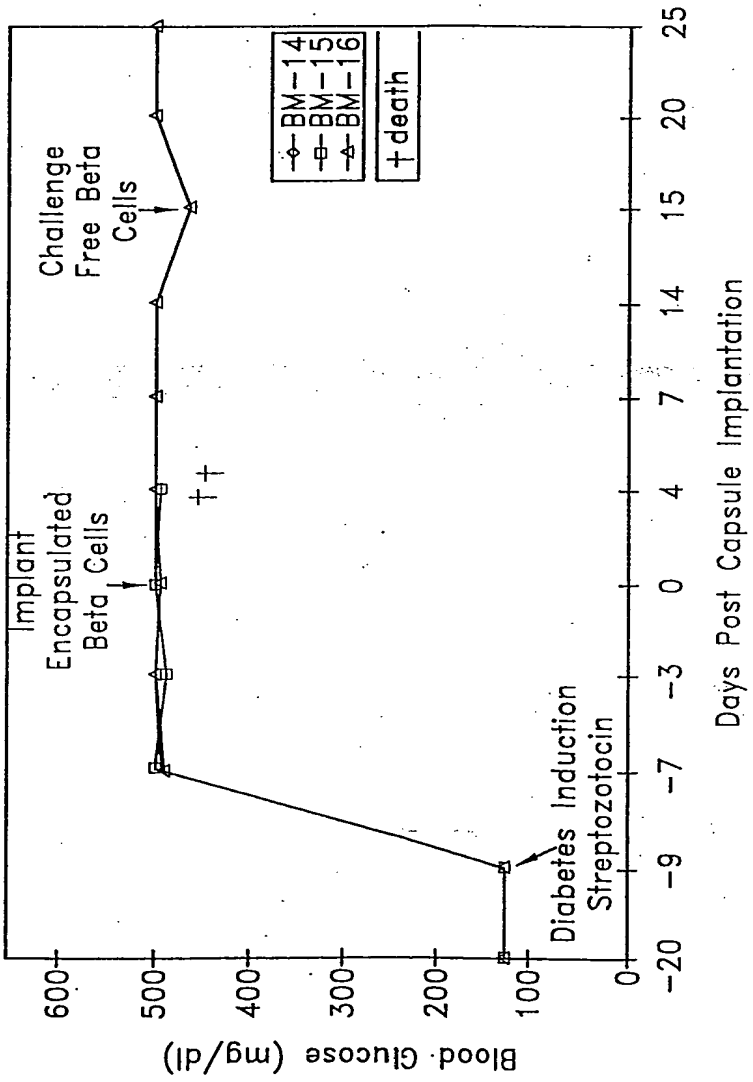
Blood Glucose (mg/dl)
600
500
400
300
200
100
0
-20
-9
-7
-3
0
4
7
14
15
20
25
Days Post Capsule Implantation
Diabetes Induction Streptozotocin
Implant Encapsulated Beta Cells
Challenge Free Beta Cells
BM-14
BM-15
BM-16
† death

FIG. 6

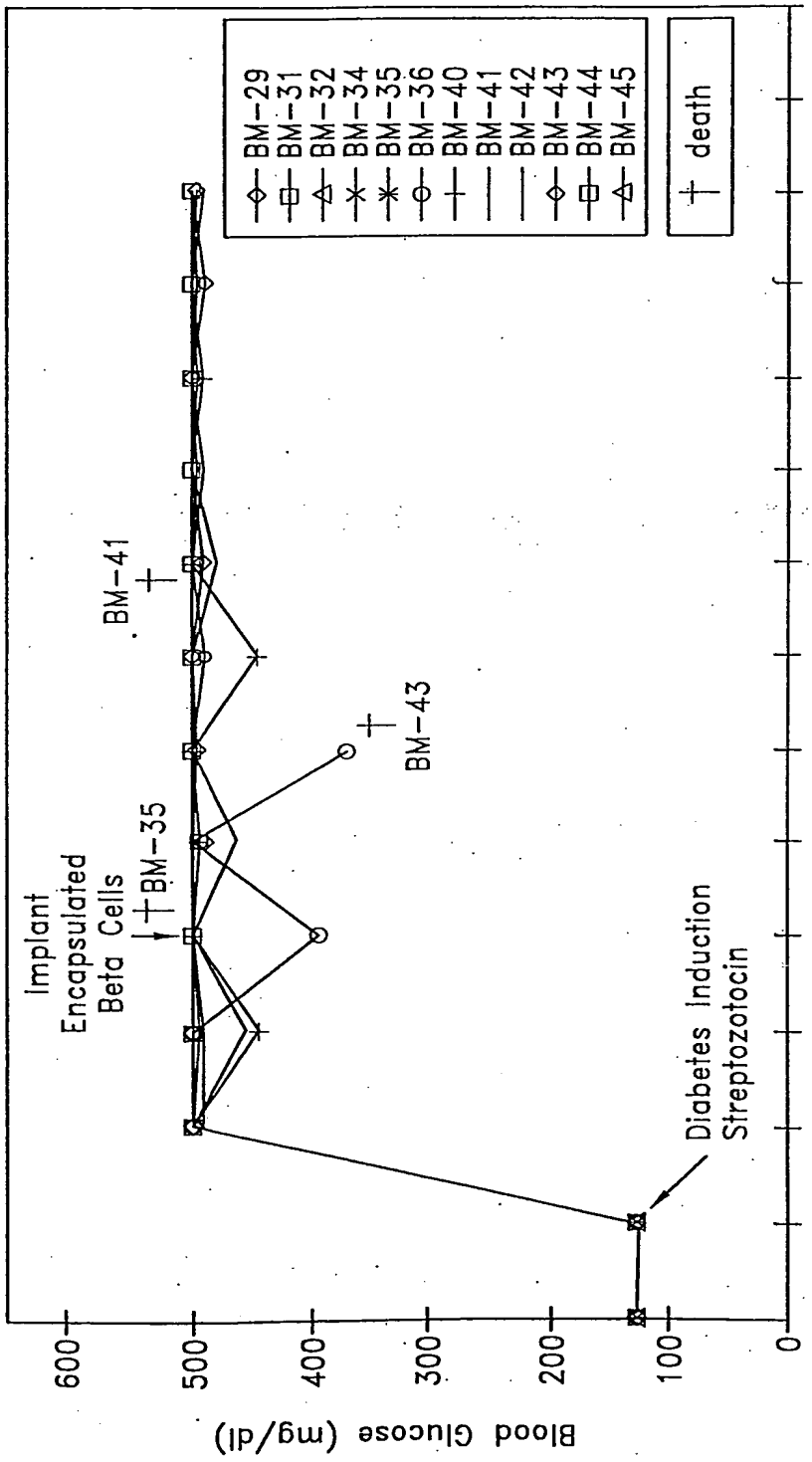
Blood Glucose (mg/dl)
600
500
400
300
200
100
0
-25
-20
-10
-2
0
1
2
3
4
5
6
7
8
9
Days Post Capsule Implantation
BM-29
BM-31
BM-32
BM-34
BM-35
BM-36
BM-40
BM-41
BM-42
BM-43
BM-44
BM-45
death
Implant Encapsulated Beta Cells
BM-35
BM-41
BM-43
Diabetes Induction Streptozotocin

FIG. 7

LATTA.002C4 MX
DEA/MXG

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BELYAVSKYI, MICHAIL A	
ART UNIT	**PAPER NUMBER**
1644	

DATE MAILED: 01/26/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)	
10/823,263	LATTA, PAUL P.	
Examiner	**Art Unit**	
Michail A Belyavskyi	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 October 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-14 are pending.

2. Applicant's election without traverses of human as spices of mammal , intraportal as species of implanting step in the reply filed on 10/12/04 is acknowledged.

Claims 1-14 read on a method of treating diabetes in mammal, wherein mammal is human, comprising implanting a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells, wherein administering is intaportal under consideration in the instant application.

3. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention *to which the claims are directed.*

4. Applicant notes that an IDS was submitted with the prior application 10/660,924. However these citations have been crossed out as said references cited in said parent application cannot be found. Applicant is invited to resubmit such references to complete the instant file. The examiner apologizes for any inconvenience to applicant for having to resubmit such documents.

5. The following is a quotation of the second paragraph of 35 U.S.C. 112.
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 1-14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. Claims 1, 6 and 11 recites the limitation "administering a curative dose" There is insufficient antecedent basis for this limitation in the claim. The preamble of the base claim 1 recites " a method of treating diabetes", not a method of curing diabetes.

B. Claim 6 is indefinite and ambiguous in the recitation of "..wherein said tolerizing and curative doses are porcine". It is unclear what Applicant means by this phrase, since " doses" can not be porcine.

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 1-14 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention commensurate in scope with this claim.

The specification disclosure does not enable one skilled in the art to practice the invention without an undue amount of experimentation.

Factors to be considered in determining whether undue experimentation is required to practice the claimed invention are summarized *In re Wands* (858 F2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). The factors most relevant to this rejection are the scope of the claim, the amount of direction or guidance provided, the limited working examples, the unpredictability in the art and the amount of experimentation required to enable one of skill in the art to practice the claimed invention.

The specification only discloses the effects of the implanting of insulin-producing cells on the level of blood glucose using streptozotocin-induced diabeties in murine experimental model. (See Examples 1-2 in particular). Examples 3-7 in the instant Specification are prophetic examples that indicate what the inventor thinks might happen in the experiments which have not actually been performed. The specification does not adequately teach how to effectively treat diabetes in mammal , wherein mammal is human, comprising implanting a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells wherein administrating step is intaportal . Moreover, the specification does not teach whether administering of second i.e. curative dose that is one or two orders of magnitude more than tolerizing dose will be tolerated (i.e. not rejected) in human. Roep et al (Nature Reviews, 2004, v.4, pages 989-997) teaches that despite more than two decades of productive research, we are still yet to define an initiating autoantigen for human disease, to determinethe precise mechanism of β-cell destruction in human and to design invention thatprevent or cure type I diabetes. Studying the pathogenesis of diabetes in human is difficult. No animal model had

been developed that shows a reproducibly high incidence of disease in a timely experimental manner. Many of the preventive therapies that seems to be promising in mouse models did not show similar efficacy in human patient. We believed that it is necessary to re-evaluate and consider at a higher level the apparent difference in the immunology and biology of diabetes between mice and men. (see entire documewnt, Abstract, pages 989, 990 and Table 1 in particular). Mestas et al (J. of Immunology, 2004, 172, pages 2731-238) teach that there exist significant differences between mice and humans in immune system development, activating and response to challenge in both the innate and adaptive arms. As therapies for human diseases become ever more sophisticated and specifically targeted it becomes increasing important to understand the potential limitations of extrapolating data from mice to humans. The literature is littered with the examples of therapies that work well in mice but fail to provide similar efficacy in humans. Teuveson et al., (Immun. Review 1993, N136, pages 101-107) teach that one problem with rodent models of transplantation is that rejection is easily overcome in said models in comparison to the difficulty of overcoming allograft rejection in human (see page 100 in particular). Teuveson et al., further teach that " however today's small animal models seem to be insufficient to produce data for clinical decision-making" and further raises doubt as to whether large animal models can be applied to clinical situations, due to species-specific reactions to treatment (see page 101 in particular). Feldman et al (Transplant. Proc. 1998, 30, 4126-4127) teach that "while it is not difficult to study the pathogenesis of animal models of disease, there are multiple constraints on analyses of the pathogenesis of human disease, leading to interesting dilemmas such as how much can we rely on and extrapolate from animal models in disease". Moreover, since the a method of treating diabetes in mammal, wherein mammal is human, comprising implanting a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells can be species- and model-dependent (see Van Noort et al. International Review of Cytology, 1998, v.178, pages 127-204, Table III in particular) , it is not clear that reliance on the *in vivo* murine data accurately reflects the relative any mammal and human efficacy of the claimed therapeutic strategy. Van Noort et al., further indicate factors that effect immune response such as genetic, environmental and hormonal (Page 176, Paragraph 3). The ability of a host to enhance an immune response will vary depending upon factors such as the condition of the host and burden of disease.

Thus, as has been discussed supra, the state of the art is that it is unpredictable form the *in vivo* murine data disclosed in the specification as whether the instant invention can be used for the *in vivo* treatment of diabetes in any mammals including human. Therefore, it is not clear that the skilled artisan could predict the efficacy a method of treating diabetes in mammal, wherein mammal is human, comprising implanting a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells. Thus in the absence of working examples or detailed guidance in the specification, the intended uses of the claimed method of treating diabetes in mammal, wherein mammal is human, comprising implanting a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells are fraught with uncertainties.

Thus, Applicant has not provided sufficient guidance to enable one skill in the art to use claimed a method of treating diabetes in mammal, wherein mammal is human, comprising implanting a tolerizing dose of insulin-secreting cells encapsulated in a biologically compatible permselective membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells in manner reasonably correlated with the scope of the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement. *In re Fisher*, 166 USPQ 18(CCPA 1970) indicates that the more unpredictable an area is, the more specific enablement is necessary in order to satisfy the statute.

In view of the quantity of experimentation necessary, the unpredictability of the art, the lack of sufficient guidance in the specification, the limited working examples, and the limited amount of direction provided given the breadth of the claims, it would take undue trials and errors to practice the claimed invention.

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 1-4 and 6 -14 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425764 or US Patent 5,629,194 each in view Posselt et al (Diabetes, 1992, v.41, pages 771-775) as is evidenced by the disclosure of Specification on overlapping pages 12-13.

US Patent '017 teaches a method of treating diabetes in a mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract and columns 6, 8, 9 –14 and Example 12 in particular) . US Patent '017 teaches that insulin producing cells are pancreatic islet cells from primary cell source (see columns 8 and 11 in particular). US Patent '017 teaches that pancreatic islet cells are from the

same species as the mammal and are implanted interperitoneally into the tissue of a mammal beneath the kidney capsule (see overlapping columns 13-14 and Example 2 in particular). US Patent '017 teaches that encapsulation of said insulin-producing cells in biologically compatible membrane for success of implantation is well known in the art (see column 12 and Example 12 in particular).

US Patent '764 teaches a method of treating diabetes in a mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract and overlapping columns 5-6 in particular). US Patent '764 teaches that insulin producing cells are pancreatic islet cells (see column 1 and 4 in particular). US Patent '764 teaches that cells are implanted interperitoneally (see column 5 in particular).

US Patent '194 teaches a method of treating diabetes in mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract overlapping columns 7-8 , 12 and Example II in particular). US Patent '764 teaches that insulin producing cells are pancreatic islet cells (see column 8 in particular). US Patent '764 teaches that cells are implanted intaportal (see column 7 in particular). US Patent '194 teaches administration of one or more anti-inflammatory agent at the dosage sufficient to achieve the desired therapeutic effect. US Patent '194 teaches that said agent can be administered prior to at the same time or subsequent to administration of insulin-producing cells (see overlapping columns 14-15 in particular).

US Patent '017 or US Patent ' 764 or US Patent '194 does not explicitly teaches a method of treating diabetes in a mammal comprising administration two doses of insulin-secreting cells one tolerizing and one curative wherein tolerizing doze is one order less than curative.

Posselet et al., teach that the important goal in the treatment of insulin-dependent diabetes by pancreatic islet transplantation is the development of strategies that allow permanent survival of pancreatic islet without continuous host immunosuppression. Posselet et al., further teach a strategy comprising two step process : first administering a small dose of cells that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site (see entire document, Abstract in particular). Posselet et al., teach that said strategy permits the survival of pancreatic islet transplant.

The Specification on overlapping pages 12-13 disclosed that curative dose is fully therapeutic dose.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of Posselt at al. to those of US Patent '017 or US Patent ' 764 or US Patent '194 to obtain a claimed method of treating diabetes in a mammal comprising

administration two doses of insulin-secreting cells one tolerizing and one curative wherein tolerizing doze is one order less than curative

One of ordinary skill in the art at the time the invention was made would have been motivated to do so, because a strategy comprising two step process : first administering a small dose of cells that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site permits the survival of pancreatic islet transplant as taught by Posselet et al. Said strategy can used in the method of treating diabetes in a mammal, comprising implanting pancreatic islet, taught by US Patent '017 or US Patent ' 764 or US Patent '194. The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination. In re Semaker. 217 USPQ 1, 5 - 6 (Fed. Cir. 1983). See MPEP 2144.

From the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 8-14 are included because it would be conventional and within the skill of the art to : (i) determine the proper pore size for the permselective membrane or (ii) to determine the optimum dosage and means of administration of insulin-secreting cells in an absent of a showing of unobvious property. Moreover, Applicant acknowledge that one of ordinary skill in the art can readily determine the proper pore size for the permselective membrane (see page 8, line 13-20 of the instant Specification in particular). Further, it has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges or means of administration involves only routine skill in the art. *In re Aller*, 220 F2d 454,456,105 USPQ 233; 235 (CCPA 1955). see MPEP § 2144.05 part II A.

11. Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425764 or US Patent 5,629,194 each in view Posselt et al (Diabetes, 1992, v.41, pages 771-775) as applied to claims 1-4 and 6-14 above, and further in view of US Patent 5,529,914.

The teaching of US Patent " 017, US Patent ' 764 , US Patent' 194 and Posselt et al., have been discussed, supra.

The combined references do not explicitly teachers a method of treating diabetes in a mammal comprising implanting insulin-secreting cells, wherein insulin-secreting cells are encapsulated in a biologically compatible membrane wherein said membrane comprises polyethylene glycol (PEG).

US Patent '914 teaches a new type of biocompatible membrane as a covering to encapsulate biological materials, comprising PEG that is acceptable for implants in mammalian. (see entire document, Abstract in particular). US Patent '914 teaches that various types of cells can be encapsulated in said biocompatible membrane and that said encapsulation will prevent rejection of encapsulated cells during transplantation (see column 10 in particular).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of US Patent '914 to those of US Patent " 017, US Patent ' 764 , US Patent' 194 and Posselt et al., to obtain a claimed method of treating diabetes in a mammal comprising implanting insulin-secreting cells, wherein insulin-secreting cells are encapsulated in a biologically compatible membrane wherein said membrane comprises polyethylene glycol (PEG).

One of ordinary skill in the art at the time the invention was made would have been motivated to do so, because encapsulation of cells in biologically compatible membrane comprising PEG will prevent rejection of encapsulated cells during transplantation as taught by US Patent '914. Said type of biocompatible membrane can be used to substitute the different type of biocompatible membrane for successful implantation of insulin-producing cells in the method of treating diabetes taught by combined references of US Patent " 017, US Patent ' 764 , US Patent' 194 and Posselt et al. The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination. In re Semaker. 217 USPQ 1, 5 - 6 (Fed. Cir. 1983). See MPEP 2144.

From the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

12. No claim is allowed.

13. The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which Applicant may become aware in the specification.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michail Belyavskyi whose telephone number is 571/272-0840. The examiner can normally be reached Monday through Friday from 9:00 AM to 5:30 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on 571/272-0841.

The fax number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Michail Belyavskyi, Ph.D.
Patent Examiner
Technology Center 1600
January 24, 2005

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FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. LATTA.002C4	APPLICATION NO. 10/823,263
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	APPLICANT PAUL P. LATTA	
(USE SEVERAL SHEETS IF NECESSARY)	FILING DATE April 13, 2004	GROUP Unknown

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	17.	A1 2,034,641	28/05/1992	CANADA				
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(USE SEVERAL SHEETS IF NECESSARY)	FILING DATE April 13, 2004	GROUP Unknown

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	APPLICANT PAUL P. LATTA	
	FILING DATE April 13, 2004	GROUP Unknown

EXAMINER INITIAL		OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)
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	Examiner Michail A Belyavskyi	Art Unit 1644	Page 1 of 2

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	C	US-5,629,194	05-1997	Dinsmore, Jonathan	435/325
	D	US-5,529,914	06-1996	Hubbell et al.	435/182
	E	US-			
	F	US-			
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	I	US-			
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*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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NON-PATENT DOCUMENTS

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✓	U	Posselt et al Diabetes, 1992, v.41, pages 771-775
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	W	
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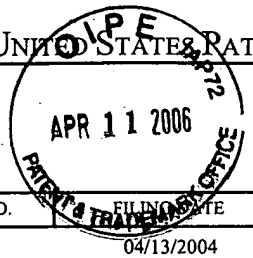

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/823,263	04/13/2004	Paul P. Latta	LATTA.002C4	3489

20995 7590 06/16/2005

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EXAMINER	
BELYAVSKYI, MICHAIL A	

ART UNIT	PAPER NUMBER
1644	

DATE MAILED: 06/16/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)	
10/823,263	LATTA, PAUL P.	
Examiner	**Art Unit**	
Michail A. Belyavskyi	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 May 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

RESPONSE TO APPLICANT'S AMENDMENT

1. Applicant's amendment, filed 05/09/05 is acknowledged.

Claims 1-14 are pending.

Claims 1-14 are under consideration in the instant application.

Applicant's amendment filed 05/09/05 in conjunction with Declaration of Dr. Scharp under 37 C.F.R 1.132 demonstrating the effectiveness of implantation of a sub-therapeutic, tolerizing dose of insulin-secreting cells and further administering an therapeutic dose of insulin-secreting cells to treat diabetes, has obviated the previous enablement rejection under 35 U.S.C. 112, first paragraph.

In view of the amendment, filed 05/09/05 the following rejections remain:

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1-4 and 6 -14 stand rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425764 or US Patent 5,629,194 each in view Posselt et al (Diabetes, 1992, v.41, pages 771-775) for the same reasons set forth in the previous Office Action, mailed on 01/26/05.

Applicant's arguments, filed 05/09/05 have been fully considered, but have not been found convincing .

Applicant asserts that: (i) US Patent '017 requires implanting only therapeutic dose of insulin-secreting cells and does not suggest implanting a tolerizing doze of insulin-producing cell prior to implanting of fully therapeutic dose; (ii) US Patent '194 does not describe or suggest implanting of a tolerizing dose of insulin producing cells prior to implanting of fully therapeutic dose; (iii) Posselt et al., teaches away from using tolerizing dose of insulin producing cells anywhere but thymus.

Applicants have traversed the primary and the secondary references pointing to the differences between the claims and the disclosure in each reference. Applicant is respectfully reminded that the rejection is under 35 USC103 and that unobviousness cannot be established by attacking the references individually when the rejection is based on the combination of the references. see In re Keller, 642 F.2d 4B, 208 USPQ 871, 882 (CCPA 1981) See MPEP 2145. This applicant has not done, but rather argues the references individually and not their combination. One cannot show non-obviousness by attacking references individually where the rejections are based on a combination of references. In re Young 403 F.2d 759, 150 USPQ 725 (CCPA 1968).

As has been stated previously, US Patent '017 teaches a method of treating diabetes in a mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract and columns 6, 8, 9 –14 and Example 12 in particular) . US Patent '017 teaches that insulin producing cells are pancreatic islet cells from primary cell source (see columns 8 and 11 in particular). US Patent '017 teaches that pancreatic islet cells are from the same species as the mammal and are implanted interperitoneally into the tissue of a mammal beneath the kidney capsule (see overlapping columns 13-14 and Example 2 in particular). US Patent '017 teaches that encapsulation of said insulin-producing cells in biologically compatible membrane for success of implantation is well known in the art (see column 12 and Example 12 in particular).

US Patent '764 teaches a method of treating diabetes in a mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract and overlapping columns 5-6 in particular). US Patent '764 teaches that insulin producing cells are pancreatic islet cells (see column 1 and 4 in particular). US Patent '764 teaches that cells are implanted interperitoneally (see column 5 in particular).

US Patent '194 teaches a method of treating diabetes in mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract overlapping columns 7-8 , 12 and Example II in particular). US Patent '764 teaches that insulin producing cells are pancreatic islet cells (see column 8 in particular). US Patent '764 teaches that cells are implanted intaportal (see column 7 in particular). US Patent '194 teaches administration of one or more anti-inflammatory agent at the dosage sufficient to achieve the desired therapeutic effect. US Patent '194 teaches that said agent can be administered prior to at the same time or subsequent to administration of insulin-producing cells (see overlapping columns 14-15 in particular).

US Patent '017 or US Patent ' 764 or US Patent '194 does not explicitly teaches a method of treating diabetes in a mammal comprising administration two doses of insulin-secreting cells one tolerizing and one curative wherein tolerizing doze is one order less than curative.

With regards to the issue that Posselt et al., teaches away from using tolerizing dose of insulin producing cells anywhere but thymus. The Examiner disagrees with Applicant's interpretation of Posselt et al. Moreover, it is noted that the instant claims does not recited any specific place where a tolerizing dose of insulin-secreting cells should be implanting. It is the Examiner position that Posselet et al., teach that the important goal in the treatment of insulin-dependent diabetes by pancreatic islet transplantation is the development of strategies that allow permanent survival of pancreatic islet without continuous host immunosuppression. Posselet et al., further teach a strategy comprising two step process : first administering a small dose of cells that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site (see entire document, Abstract in particular). Posselet et al., teach that said strategy permits the survival of pancreatic islet transplant.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of Posselt at al. to those of US Patent '017 or US Patent ' 764 or US Patent '194 to obtain a claimed method of treating diabetes in a mammal comprising administration two doses of insulin-secreting cells one tolerizing and one curative wherein tolerizing doze is one order less than curative

One of ordinary skill in the art at the time the invention was made would have been motivated to do so, because a strategy comprising two step process : first administering a small dose of cells that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site permits the survival of pancreatic islet transplant as taught by Posselet et al. Said strategy can used in the method of treating diabetes in a mammal, comprising implanting pancreatic islet, taught by US Patent '017 or US Patent ' 764 or US Patent '194. The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination. In re Semaker. 217 USPQ 1, 5 - 6 (Fed. Cir. 1983). See MPEP 2144.

From the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 8-14 are included because it would be conventional and within the skill of the art to : (i) determine the proper pore size for the permselective membrane or (ii) to determine the optimum dosage and means of administration of insulin-secreting cells in an absent of a showing of unobvious property. Moreover, Applicant acknowledge that one of ordinary skill in the art can readily determine the proper pore size for the permselective membrane (see page 8, line 13-20

of the instant Specification in particular). Further, it has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges or means of administration involves only routine skill in the art. *In re Aller*, 220 F2d 454,456,105 USPQ 233; 235 (CCPA 1955). see MPEP § 2144.05 part II A.

4. Claim 5 stands rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425764 or US Patent 5,629,194 each in view Posselt et al (Diabetes, 1992, v.41, pages 771-775) as applied to claims 1-4 and 6-14 above, and further in view of US Patent 5,529,914 for the same reasons set forth in the previous Office Action, mailed on 01/26/05.

Applicant's arguments, filed 05/09/05 have been fully considered, but have not been found convincing .

Applicant asserts that because US Patent 6,703,017, US Patent 5,425764 US Patent 5,629,194 and Posselt et al., are not prior art and do not suggest the claimed invention they can not be used in combination with US Patent 5,529,914.

As have been discussed, supra, it is the Examiner position that the prior art of US Patent 6,703,017, US Patent 5,425764 US Patent 5,629,194 and Posselt et al., do suggest the claimed invention and thus can be used in combination with US Patent 5,529,914.

The combined references do not explicitly teachers a method of treating diabetes in a mammal comprising implanting insulin-secreting cells, wherein insulin-secreting cells are encapsulated in a biologically compatible membrane wherein said membrane comprises polyethylene glycol (PEG).

US Patent '914 teaches a new type of biocompatible membrane as a covering to encapsulate biological materials, comprising PEG that is acceptable for implants in mammalian. (see entire document, Abstract in particular). US Patent '914 teaches that various types of cells can be encapsulated in said biocompatible membrane and that said encapsulation will prevent rejection of encapsulated cells during transplantation (see column 10 in particular).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of US Patent '914 to those of US Patent " 017, US Patent ' 764 , US Patent' 194 and Posselt et al., to obtain a claimed method of treating diabetes in a mammal comprising implanting insulin-secreting cells, wherein insulin-secreting cells are encapsulated in a biologically compatible membrane wherein said membrane comprises polyethylene glycol (PEG).

One of ordinary skill in the art at the time the invention was made would have been motivated to do so, because encapsulation of cells in biologically compatible membrane comprising PEG will prevent rejection of encapsulated cells during transplantation as taught by US Patent '914. Said type of biocompatible membrane can be used to substitute the different type of biocompatible membrane for successful implantation of insulin-producing cells in the method of treating diabetes taught by combined references of US Patent " 017, US Patent '764 , US Patent' 194 and Posselt et al. The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination. In re Semaker. 217 USPQ 1, 5 - 6 (Fed. Cir. 1983). See MPEP 2144.

From the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

5. No claim is allowed.

6. **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michail Belyavskyi whose telephone number is 571/ 272-0840 The examiner can normally be reached Monday through Friday from 9:00 AM to 5:30 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on 571/ 272-0841 .

The fax number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Michail Belyavskyi, Ph.D.
Patent Examiner
Technology Center 1600
June 6, 2005

CHRISTINA CHAN
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

LATTA.002C4
DEA/MXG

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/823,263	04/13/2004	Paul P. Latta	LATTA.002C4	3489

20995 7590 11/03/2005

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EXAMINER	
BELYAVSKYI, MICHAIL A	
ART UNIT	**PAPER NUMBER**
1644	

DATE MAILED: 11/03/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)	
10/823,263	LATTA, PAUL P.	
Examiner	**Art Unit**	
Michail A. Belyavskyi	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 August 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11,13 and 14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11,13 and 14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 08/19/05 has been entered.

Claims 1-11, 13-14 are pending.

Claims 1-11, 13-14 are under consideration in the instant application.

In view of the amendment, filed 08/19/05 the following rejections remain:

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1-4 and 6 –11,13 and 14 stand rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425764 or US Patent 5,629,194 each in view Posselt et al (Diabetes, 1992, v.41, pages 771-775) for the same reasons set forth in the previous Office Action, mailed on 06/16/05

Applicant's arguments, filed 08/19/05 have been fully considered, but have not been found convincing .

Applicant asserts that Posselt et al., teaches away from using tolerizing dose of insulin producing cells anywhere but thymus and it only shows success in the absence of prior sensitization to the implant.

The Examiner disagrees with Applicant's interpretation of Posselt et al. The issue raised in the previous Office Action was that it is the Examiner position that Posselet et al., teach two step stratergy: first administering a small dose of cells that induces an unresponsive state, i.e.

tolerizing dose and then administering fully therapeutic dose, at another site. Posselet et al., teach that said strategy permits the survival of pancreatic islet transplant. (see entire document, Abstract and page365 in particular). The fact that Posselet et al., implanted the first, i.e. tolerizing dose into thymus does not neglect the teaching of the advantages of using the two step process i.e. implanting first a small number of cells in one place and then implanting a therapeutic dose in different place. Posselet et al., teach that the important goal in the treatment of insulin-dependent diabetes by pancreatic islet transplantation is the development of strategies that allow permanent survival of pancreatic islet without continuous host immunosuppression. There is no indication or suggestion in Posselt et al. that only intrathymic transplantation should be performed. Posselt et al., teach that the finding that recipient bearing established intrathymic graft fail to destroy subsequent extrathymic islets either by rejection or autoimmunity argues that additional mechanism that alter systemic immune response are also involved. In other words, one skilled in the art would immediately recognized that Posselet et al., teach an advantage of two step process in the treatment of insulin-dependent diabetes by pancreatic islet transplantation. However, it is noted that the instant claims does not recited any specific place where a first tolerizing dose of insulin-secreting cells should be implanting.

US Patent '017 teaches a method of treating diabetes in a mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract and columns 6, 8, 9 –14 and Example 12 in particular) . US Patent '017 teaches that insulin producing cells are pancreatic islet cells from primary cell source (see columns 8 and 11 in particular). US Patent '017 teaches that pancreatic islet cells are from the same species as the mammal and are implanted interperitoneally into the tissue of a mammal beneath the kidney capsule (see overlapping columns 13-14 and Example 2 in particular). US Patent '017 teaches that encapsulation of said insulin-producing cells in biologically compatible membrane for success of implantation is well known in the art (see column 12 and Example 12 in particular).

US Patent '764 teaches a method of treating diabetes in a mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract and overlapping columns 5-6 in particular). US Patent '764 teaches that insulin producing cells are pancreatic islet cells (see column 1 and 4 in particular). US Patent '764 teaches that cells are implanted interperitoneally (see column 5 in particular).

US Patent '194 teaches a method of treating diabetes in mammal comprising implanting insulin-producing cells encapsulated in a biologically compatible membrane (see entire document, Abstract overlapping columns 7-8 , 12 and Example II in particular). US Patent '764 teaches that insulin producing cells are pancreatic islet cells (see column 8 in particular). US Patent '764 teaches that cells are implanted intaportal (see column 7 in particular). US Patent '194 teaches administration of one or more anti-inflammatory agent at the dosage sufficient to achieve the desired therapeutic effect. US Patent '194 teaches that said agent can be administered prior to

at the same time or subsequent to administration of insulin-producing cells (see overlapping columns 14-15 in particular).

US Patent '017 or US Patent ' 764 or US Patent '194 does not explicitly teaches a method of treating diabetes in a mammal comprising administration two doses of insulin-secreting cells one tolerizing and one curative wherein tolerizing doze is one order less than curative.

As has been discuused supra, it is the Examiner position that Posselet et al., teach that the important goal in the treatment of insulin-dependent diabetes by pancreatic islet transplantation is the development of strategies that allow permanent survival of pancreatic islet without continuous host immunosuppression. Posselet et al., further teach a strategy comprising two step process : first administering a small dose of cells that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site (see entire document, Abstract in particular). Posselet et al., teach that said strategy permits the survival of pancreatic islet transplant.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of Posselt at al. to those of US Patent '017 or US Patent ' 764 or US Patent '194 to obtain a claimed method of treating diabetes in a mammal comprising administration two doses of insulin-secreting cells one tolerizing and one curative wherein tolerizing doze is one order less than curative

One of ordinary skill in the art at the time the invention was made would have been motivated to do so, because a strategy comprising two step process : first administering a small dose of cells that induces an unresponsive state, i.e. tolerizing dose and then administering fully therapeutic dose, at another site permits the survival of pancreatic islet transplant as taught by Posselet et al. Said strategy can used in the method of treating diabetes in a mammal, comprising implanting pancreatic islet, taught by US Patent '017 or US Patent ' 764 or US Patent '194. The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination. In re Semaker. 217 USPQ 1, 5 - 6 (Fed. Cir. 1983). See MPEP 2144.

From the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Claims 8-11, 13 and 14 are included because it would be conventional and within the skill of the art to : (i) determine the proper pore size for the permselective membrane or (ii) to determine the optimum dosage and means of administration of insulin-secreting cells in an absent of a showing of unobvious property. Moreover, Applicant acknowledge that one of ordinary skill in the art can readily determine the proper pore size for the permselective membrane (see page 8, line 13-20 of the instant Specification in particular). Further, it has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges or means of administration involves only routine skill in the art. *In re Aller*, 220 F2d 454,456,105 USPQ 233; 235 (CCPA 1955). see MPEP § 2144.05 part II A.

4. Claim 5 stands rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425764 or US Patent 5,629,194 each in view Posselt et al (Diabetes, 1992, v.41, pages 771-775) as applied to claims 1-4 and 6-14 above, and further in view of US Patent 5,529,914 for the same reasons set forth in the previous Office Action, mailed on 06/16/05

Applicant's arguments, filed 08/19/05 have been fully considered, but have not been found convincing .

Applicant asserts that because US Patent 6,703,017, US Patent 5,425764 US Patent 5,629,194 and Posselt et al., are not prior art and do not suggest the claimed invention they can not be used in combination with US Patent 5,529,914.

As have been discussed, supra, it is the Examiner position that the prior art of US Patent 6,703,017, US Patent 5,425764 US Patent 5,629,194 and Posselt et al., do suggest the claimed invention and thus can be used in combination with US Patent 5,529,914.

The combined references do not explicitly teachers a method of treating diabetes in a mammal comprising implanting insulin-secreting cells, wherein insulin-secreting cells are encapsulated in a biologically compatible membrane wherein said membrane comprises polyethylene glycol (PEG).

US Patent '914 teaches a new type of biocompatible membrane as a covering to encapsulate biological materials, comprising PEG that is acceptable for implants in mammalian. (see entire document, Abstract in particular). US Patent '914 teaches that various types of cells can be encapsulated in said biocompatible membrane and that said encapsulation will prevent rejection of encapsulated cells during transplantation (see column 10 in particular).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to apply the teaching of US Patent '914 to those of US Patent " 017, US Patent ' 764 , US Patent' 194 and Posselt et al., to obtain a claimed method of treating diabetes in a mammal comprising implanting insulin-secreting cells, wherein insulin-secreting cells are encapsulated in a biologically compatible membrane wherein said membrane comprises polyethylene glycol (PEG).

One of ordinary skill in the art at the time the invention was made would have been motivated to do so, because encapsulation of cells in biologically compatible membrane comprising PEG will prevent rejection of encapsulated cells during transplantation as taught by US Patent '914. Said type of biocompatible membrane can be used to substitute the different type of biocompatible membrane for successful implantation of insulin-producing cells in the method of treating diabetes taught by combined references of US Patent " 017, US Patent ' 764 , US Patent' 194 and Posselt et al. The strongest rationale for combining references is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination. In re Semaker. 217 USPQ 1, 5 - 6 (Fed. Cir. 1983). See MPEP 2144.

From the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

5. No claim is allowed.

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michail Belyavskyi whose telephone number is 571/272-0840. The examiner can normally be reached Monday through Friday from 9:00 AM to 5:30 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on 571/272-0841.

The fax number for the organization where this application or proceeding is assigned is 571/273-8300

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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Intrathymic Islet Transplantation in the Spontaneously Diabetic BB Rat

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Recently it was demonstrated that pancreatic islet allografts transplanted to the thymus of rats made diabetic chemically are not rejected and induce specific unresponsiveness to subsequent extrathymic transplants. The authors report that the thymus can also serve as an effective islet transplantation site in spontaneously diabetic BB rats, in which autoimmunity and rejection can destroy islets. Intrathymic Lewis islet grafts consistently reversed hyperglycemia for more than 120 days in these rats, and in three of four recipients the grafts promoted subsequent survival of intraportal islets. In contrast intraportal islet allografts in naive BB hosts all failed rapidly. The authors also show that the immunologically privileged status of the thymus cannot prevent rejection of islet allografts in Wistar Furth (WF) rats sensitized with donor strain skin and that suppressor cells are not likely to contribute to the unresponsive state because adoptive transfer of spleen cells from WF rats bearing established intrathymic Lewis islets fails to prolong islet allograft survival in secondary hosts.

THE FAILURE OF currently available immunosuppressive protocols to prolong the survival of pancreatic islet allografts has prompted a search for alternate methods that can permit long-term graft function without the need for chronic immunosuppression. One strategy used with some success in animal systems is the transplantation of endocrine tissue to natural or artificially constructed immunologically privileged sites. While many of the best described privileged sites, such as the anterior chamber of the eye and alymphatic skin flap, are incapable of supporting islet endocrine function, others, such as the cerebral cortex and abdominally displaced testicle, have been shown to permit survival of implanted islets and in addition to protect them from alloimmune rejection.[1-3] Although these techniques deserve consideration because they obviate the need for host immunosuppression or pretransplant immunomodulation of the graft, practical considerations would limit their use in clinical islet transplantation.

Recently we reported that the thymus can serve as a novel transplant site that provides a suitable environment for islet endocrine function and promotes survival of allografts without chronic immunosuppression of the recipient. Furthermore animals bearing established intrathymic islet grafts are rendered specifically unresponsive to donor alloantigens as demonstrated by their inability to reject donor-strain islets transplanted to an extrathymic site.[4]

In the present study, we have evaluated further the mechanisms responsible for prolonged survival of intrathymic grafts and for the induction of specific tolerance that follows intrathymic islet transplantation. In addition we have determined the outcome of intrathymic islet transplantation in spontaneously diabetic BB rats, in which both rejection and recurrent anti-beta cell autoimmunity can destroy islet allografts.[5-7]

Materials and Methods

Animals

BB rats were bred and maintained in the animal facilities of the University of Pennsylvania. In our subline of diabetes-prone rats, spontaneous diabetes has a cumulative incidence of 50% to 60% and develops between 60 to 120 days of age. Rats that were considered to have

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developed diabetes (serum glucose levels more than 300 mg/dL on 3 consecutive days) received daily injections of protamine zinc insulin to prevent ketoacidosis and death. Insulin therapy was discontinued after islet transplantation so that blood glucose levels could serve as an index of islet graft survival. Spontaneous recovery from diabetes in BB rats has never occurred in our colony. All BB rats are homozygous for the $RT1^u$ haplotype at the major histocompatibility complex (MHC) locus.

Inbred Wistar Furth (WF, $RT1^u$) and Lewis ($RT1^l$) rats were purchased from Harlan-Sprague Dawley (Walkersville, MD) and Charles River Laboratories (Wilmington, MA), respectively.

Induction of Chemical Diabetes

Hyperglycemia was induced in nondiabetic WF rats by a single intravenous injection (65 mg/kg) of streptozotocin. Only rats with nonfasting blood glucose levels of more than 300 mg/dL were considered suitable for transplantation with pancreatic islets.

Islet Isolation and Transplantation

Islets were isolated by collagenase digestion of the pancreas followed by centrifugation through a discontinuous Ficoll gradient as previously described.[8] Contaminating nonislet tissue was removed under a dissecting microscope, and only islets free of adherent acinar, vascular, and lymphoid elements were used for transplantation. Spontaneously diabetic animals received islet grafts 2 to 7 days after onset of diabetes, while chemically induced diabetic rats were allowed to remain hyperglycemic for 6 to 14 days to exclude the possibility that insufficient streptozotocin had been administered to cause permanent diabetes. Freshly isolated islets (1000 to 1500) were transplanted into the following sites: (1) the liver *via* portal vein embolization, (2) the renal subcapsule, (3) the abdominally displaced testicle, and (4) the thymus by injection of 600 to 800 islets into each lobe. Islets were never maintained in tissue culture before transplantation. Hyperglycemia usually was reversed within 1 day and always within 4 days after transplantation. Where noted recipients received 1 mL of rabbit anti-rat lymphocyte serum (ALS) (Accurate Chemical and Scientific Co., Westbury, NY) intraperitoneally at the time of transplantation. Nonfasting blood glucose levels were determined three times each week in all islet recipients, with cessation of graft function being defined as the first of 2 consecutive days of recurrent hyperglycemia (blood glucose level more than 200 mg/dL). At the conclusion of each study, animals were killed and islet-bearing organs were removed, fixed in Bouin's solution, and processed for light microscopy. Serial sections of each organ were stained with hematoxylin and eosin as well as with aldehyde-fuchsin for identification of islet tissue.

Monoclonal Antibodies (MAb) and Flow Cytometry

The following murine mAbs specific for rat lymphocyte markers were used: R73 (anti-α/β T-cell receptor [TCR]; all T cells), OX-8 (anti-CD8; cytolytic/suppressor T cells and NK cells), W3/25 (anti-CD4; helper T cells and macrophages), and Mar 18.5 (anti-surface immunoglobulin, (sIg); B cells). The antibodies were characterized previously.[9,10] Monoclonal reagents were used either as undiluted culture supernatants or obtained in purified form from Bioproducts for Science, Inc. (Indianapolis, IN).

For immunofluorescent analysis, 1 to 2×10^6 cervical and mesenteric lymph node cells (LNC) were incubated for 60 minutes with saturating concentrations of the primary mAb, washed twice, and treated for 60 minutes with fluorescein isothiocyanate-conjugated $F(ab')_2$ goat anti-mouse IgG (Tago, Inc., Burlingame, CA). All incubations were performed at 4 C in Dulbecco's phosphate-buffered saline (D-PBS) containing 0.01% sodium azide. Ten thousand viable cells were analyzed for relative fluorescence using a dual laser fluorescence-activated cell sorter (FACS) with logarithmic amplifiers (FACS IV; Becton-Dickinson, Sunnyvale, CA). Background fluorescence was calculated using cells incubated with the fluorescein conjugate alone.

Adoptive Transfer Studies

Spleens from long-term (more than 200 days) WF recipients of intrathymic Lewis islet allografts were teased into single-cell suspensions, washed twice in D-PBS, and injected intravenously (250 to 300 $\times$ 10^6 cells/recipient) into sublethally irradiated (450 R), streptozotocin-diabetic WF rats. Twenty-four hours later, animals received fresh Lewis islet allografts beneath the renal capsule without immunosuppression. Control animals received spleen cells from unmanipulated WF rats.

Skin Grafting

Skin grafting was performed according to the method of Billingham.[11] Rejection was defined by extensive graft necrosis and sloughing, as judged by daily inspection after dressings were removed on the seventh day.

Results

Survival of Islet Allografts in Nonimmunosuppressed Spontaneously Diabetic BB Rats

Pancreatic islets isolated from Lewis donors and transplanted by portal vein inoculation into six spontaneously diabetic BB rats were destroyed promptly in every instance (median survival time [MST], 9 days). In this model it is

1,000 1,500 islets $\times$ 1000 cells/rat produced normoglycemia

140-(250)

TABLE 1. *Survival of MHC-compatible and -incompatible Islet Allografts in BB Rats*

Transplant Site	Donor Strain (MHC)	Days of Allograft Survival (MST)*
Liver (intraportal)	Lewis ($RT1^l$)	8, 9, 9, 19, 24, (9)*
Renal subcapsule	Lewis ($RT1^l$)	41, 47, 59, >70† >120 × 2 (>64.5)
	WF ($RT1^u$)	23, 64, >120 (64)
Thymus	Lewis ($RT1^l$)	>50 × 5, >120 × 6 (>120)‡
	WF ($RT1^u$)	>120 × 5 (>120)

impossible to distinguish rejection from autoimmune damage, which in itself can cause islet failure. As we have found previously, islets transplanted beneath the renal capsule had a more prolonged and variable survival in BB rats, which are known to be significantly immunodeficient.[12,13] Of the 6 recipients of renal subcapsular grafts, 3 underwent islet failure after 41, 47, and 59 days, 1 died while still normoglycemic after 70 days, and 2 others remained normoglycemic for more than 120 days before they were used for other experiments (see below). In contrast to the results in other transplant sites, none of 11 BB rats that received intrathymic Lewis islets destroyed their grafts. Serum glucose levels in these animals returned to normal within 48 to 72 hours after islet transplantation and remained between 80 and 110 mg/dL for the duration of the study. Six of these animals, after being followed for more than 120 days, were then studied histologically and/or used for other experiments. Five other normoglycemic animals are still being followed for periods between 50 and 67 days. These findings are summarized in Table 1.

If autoimmune diabetes is an MHC-restricted process, islet allografts from MHC-compatible donors, although less susceptible to rejection by BB recipients than those from MHC-incompatible donors, would be more likely to be destroyed by autoimmunity.[14,15] Therefore to investigate whether the thymus protects MHC-compatible islet grafts from rejection and, more importantly, also from autoimmune damage, we compared the outcome of intrathymic and renal subcapsular grafts of WF islets in spontaneously diabetic BB rats. All five recipients of intrathymic WF islets maintained normal serum glucose levels for more than 120 days, while two of three recipients of renal subcapsular WF grafts became hyperglycemic after 23 and 64 days (Table 1).

Assessment of Unresponsiveness in Islet Allograft Recipients

To determine whether intrathymic islets transplanted to diabetic BB rats would induce an unresponsive state that could protect subsequent extrathymic islets from either rejection or autoimmunity, five BB rats that had harbored an intrathymic Lewis islet transplant for 120 days received another Lewis islet allograft transplanted intraportally. No immunosuppression was given at any time. All animals remained normoglycemic and 60 to 65 days after the second allograft the thymus containing the first allograft was removed in four of these rats to allow functional assessment of the extrathymic islet transplant (Fig. 1). Three of the four animals remained normoglycemic after thymectomy while one (BB#1) became hyperglycemic (blood glucose level, 250 to 300 mg/dL) although it remained healthy and continued to gain weight, unlike

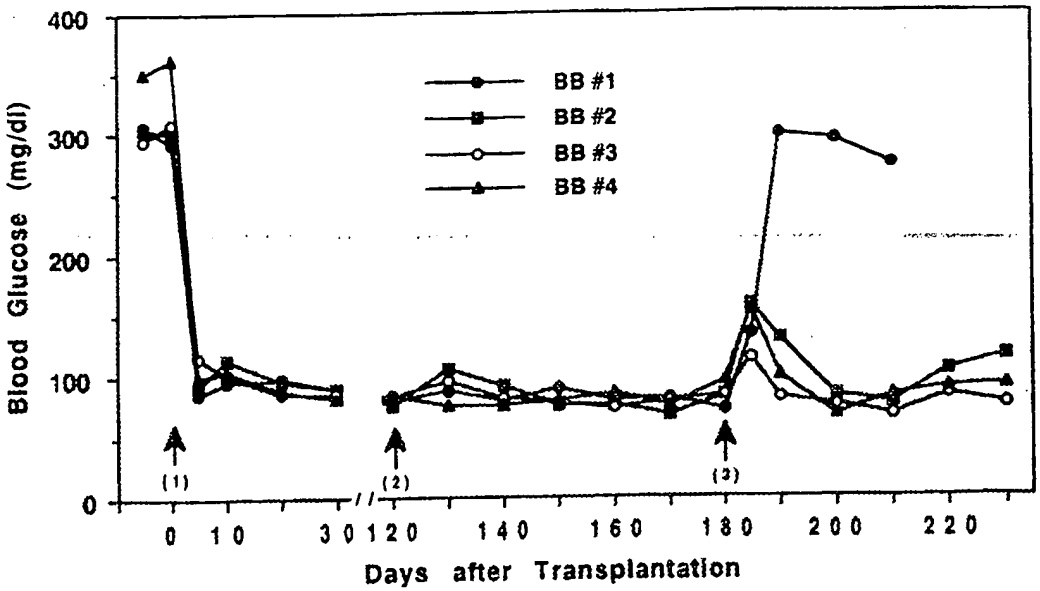

FIG. 1. Blood glucose profiles of spontaneously diabetic BB rats (BB # 1-4) transplanted with Lewis islet allografts. (1) Intrathymic islet transplantation; (2) second transplantation of Lewis islets intraportally; (3) removal of islet-bearing thymus.

untreated BB rats, which usually do not survive without exogenous insulin treatment.

In all six recipients of intrathymic Lewis islets, histologic examination of the thymus after 120 days revealed healthy, well-granulated islets and no lymphocytic infiltrate. In the animals that had remained normoglycemic after thymectomy, the liver, when finally examined histologically, also contained well-granulated noninfiltrated islets (Fig. 2). The liver of the one rat that became hyperglycemic after thymectomy contained intact islets with

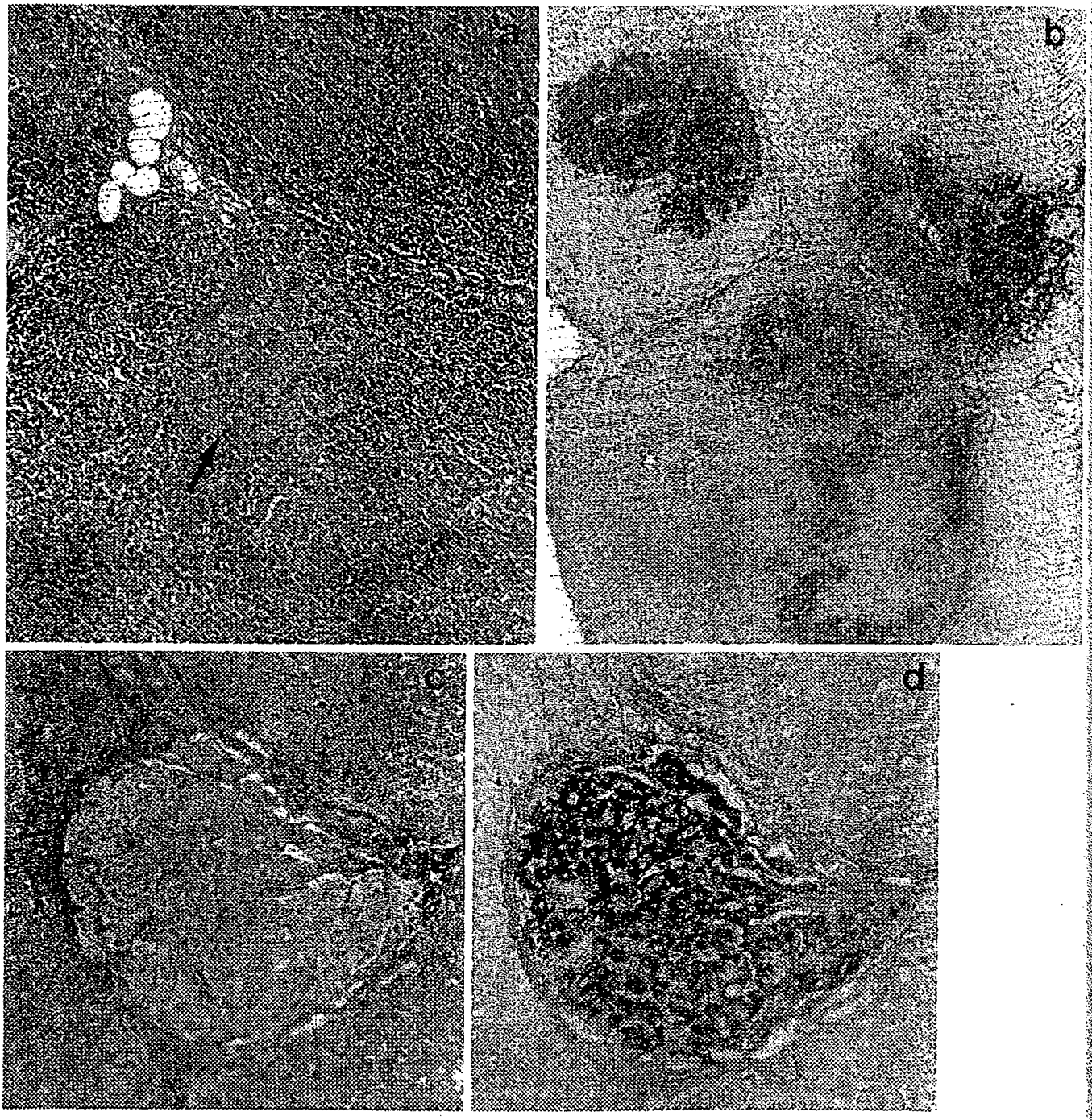

FIG. 2. Photomicrographs of thymus and liver from BB recipients of Lewis islet allografts. (a) Section of a thymus removed 180 days after implantation of Lewis islets. The islets (arrow) appear healthy and there is no mononuclear infiltration (H&E ×80). (b) Aldehyde-fuchsin–stained section from the same specimen demonstrating abundant insulin granules (×20). (c) Section of liver from a rat that was transplanted with intraportal Lewis islets 120 days after intrathymic islet transplantation. Removal of the liver was performed 110 days after intraportal islet transplantation (H&E ×160). (d) The presence of beta cells in the same section is indicated by insulin-specific staining (aldehyde-fuchsin ×160).

no suggestion of insulitis; however they stained weakly for insulin. This histologic finding suggested that an insufficient number of islets had been transplanted to the liver to allow the intraportal graft alone to maintain normoglycemia after removal of the thymus. Such grafts are likely to show degranulation of beta cells.[16]

Although most renal subcapsular transplants in BB rats eventually fail, in this study two rats maintained functional kidney subcapsular islets for more than 120 days, allowing us to assess the capacity of allografts in this site to induce unresponsiveness. One of these rats was grafted with Lewis skin, which was rejected after 29 days, provoking rejection of the islet graft 9 days later. The other rat received a second Lewis islet allograft, transplanted intraportally. This animal remained normoglycemic, and 60 days later was killed for histologic examination of the transplanted islets. Although noninfiltrated islets were found under the renal capsule, only a few recognizable islets remained in the liver, and these were infiltrated by mononuclear cells. Thus it appeared that the persistence of allogeneic islets in the kidney does not diminish the vigor of the immune response to subsequent allografts of donor-strain skin or extrathymic islets. The pancreata of all animals mentioned above were examined histologically and in no instance did recognizable beta cells remain.

Studies of Intrathymic Islet Allografts in Immunized Hosts

To evaluate the capacity of the thymus to act as a privileged site in presensitized hosts, four WF rats were sensitized with Lewis skin allografts. After rejection of the skin grafts, the rats were rendered diabetic with streptozotocin and 5 days later transplanted with Lewis islets into either the thymus or another privileged site (the testicle), in conjunction with 1 mL of ALS administered intraperitoneally. All animals became normoglycemic after transplantation but rejected the islets rapidly (within 10 days), indicating that the efferent arc of the immune response is intact for both intratesticular and intrathymic grafts and confirming our previous experience with allografts in other privileged sites (Table 2).[17]

The vulnerability of established intrathymic islet allografts to immunity induced by subsequent donor strain skin also was assessed. Three (two WF, one BB) rats that had maintained functional intrathymic Lewis islet allografts for 120 to 200 days were grafted with Lewis skin. Skin allograft survival in the two WF rats was minimally if at all prolonged (11, 14 days *versus* 9 to 10 days in six controls). This was also the finding in the BB recipient, in which rejection of the skin was complete in 30 days (control MST: 32 days, n = 4). Interestingly none of these animals rejected the skin grafts in an accelerated manner, indicating that they had not been sensitized by the intrathymic islets and suggesting interruption in the afferent arc of the immune response. The fate of the long-standing intrathymic islets was variable in these same animals after they were challenged with a skin allograft. In the BB rat, diabetes recurred 36 days after skin grafting (6 days after the completion of skin graft rejection). One of the WF rats became hyperglycemic 11 days after skin grafting, while the other has remained normoglycemic despite rejection of the donor-strain skin allograft.

TABLE 2. *Survival of Lewis Islet Allografts in Sensitized WF Recipients**

Transplant Site	Days of Allograft Survival	
	Sensitized Hosts*	Unsensitized Hosts
Testicle	10, 10	50 × 2, 76, 110, >200 × 2
Thymus	5, 6	28, 33, 57, >200 × 10

† All recipients received 1 cc ALS administered intraperitoneally at time of islet transplantation.

* Islets were transplanted 5 days after rejection of a Lewis skin allograft.

In Vitro *Assays to Characterize the Unresponsive State Induced by Intrathymic Islet Allografts*

Several assays were performed to define possible differences in the immune system induced by the presence of alien antigen in the thymus. To exclude the possibility that inoculation of islets into the thymus had disrupted T-cell maturation and thus rendered the animals immunodeficient, lymph node cells from long-term (more than 120 days) BB or WF recipients of intrathymic islets were analyzed by flow cytometry (Fig. 3). Cells from unmanipulated and intrathymically grafted WF rats showed similar percentages of TCR+, CD8+, CD4+, and sIg+ subsets. While both naive and transplanted BB rats demonstrated the T-cell lymphopenia characteristic of this strain,[18] no significant phenotypic differences were noted between these two groups.

Mixed lymphocyte culture assays previously performed on LNC from WF recipients of intrathymic Lewis islets showed normal proliferative responses to donor alloantigens as compared to nontransplanted controls.[4] We did not perform these assays in BB rats because there is never a significant *in vitro* proliferative response to allogeneic stimulators.[18,19]

Adoptive Transfer Studies to Assess Possible Suppressor Cell Activity in Intrathymic Islet Recipients

Two mechanisms that could account for the unresponsiveness of long-term recipients of intrathymic allogeneic islets to subsequent extrathymic transplants are (1) deletion or inactivation within the thymus of specific T-cell populations that could otherwise be expected to destroy extrathymic grafts and (2) generation of suppressor T cells.

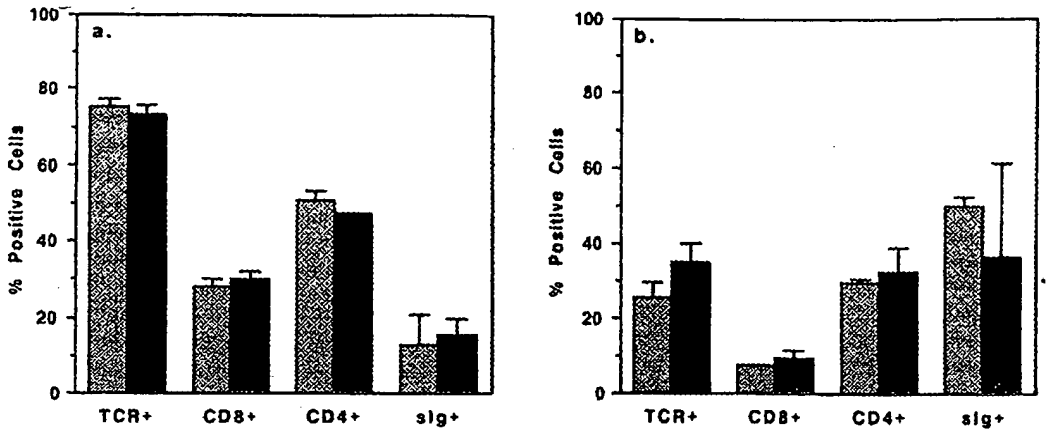

FIG. 3.. Immunofluorescent analysis of lymph node cells from WF and BB rats bearing long-term (more than 120 days) intrathymic (IT) Lewis islet allografts. (a) WF controls (shaded bars); WF recipients of intrathymic islets (solid bars). (b) untransplanted spontaneously diabetic BB controls (shaded bars); BB recipients of intrathymic islets (solid bars). Results are means ± SD of three or four separate experiments.

We have already reported the results of studies to assess the first possibility (see Discussion). To evaluate the latter, we adoptively transferred 250 to 300 × 10^6 spleen cells either from nontransplanted WF controls or from WF rats harboring intrathymic Lewis islet allografts for more than 100 days to sublethally irradiated WF hosts. These animals subsequently received islets from Lewis donors beneath the renal capsule. Islet survival in rats given spleen cells from intrathymic recipients was not significantly different than that of the control group and thus provided no evidence for suppressor cells (Table 3).

Discussion

Recently we found the thymus to be another of the relatively few transplant sites that will permit successful engraftment and normal metabolic function of pancreatic islets. Even more interesting was the concomitant observation that the thymus is a previously unrecognized immunologically privileged site. Islet allografts transplanted intrathymically to nonimmunosuppressed rats with chemically induced diabetes exhibited substantially prolonged survival and, if the hosts were briefly immunosuppressed with a single dose of ALS, permanent allograft survival was achieved. Although the precise mechanisms responsible for these findings remain to be elucidated, a partial explanation is suggested by several known morphologic and physiologic characteristics of the organ. Ultrastructural studies as well as experiments using particulate dyes have demonstrated the presence of a blood–thymus barrier surrounding capillaries in the thymic cortex.[20] In addition the thymus possesses no afferent lymphatic supply.[21] These details of vascular anatomy may account for the findings of Michie et al.[22] that there is minimal recirculation of mature T lymphocytes through the thymic parenchyma. Despite the classification of the thymus as a primary lymphoid organ, it is relatively removed from the immune surveillance, that takes place in other tissues, thus explaining the sanctuary it provides for allografts.

In the present study, we sought to determine whether the thymus' immunologically privileged nature would be sufficient to sustain allografts implanted in sensitized hosts that bear an expanded population of effector T cells reactive to donor alloantigens. However, when we transplanted allogeneic islets into the thymus of recipients that had previously rejected donor strain skin grafts, the islets were destroyed in an accelerated manner, demonstrating that the intrathymic site is readily accessible at least to activated T cells. These findings correlate with reports that antigen-stimulated T lymphocytes have an enhanced capacity to recirculate through the thymic parenchyma.[23,24] That the thymus is indeed an immunologically privileged site is not contradicted by these results because it is well established that even sites of proved immunologic privilege such as the anterior chamber of the eye, the brain, and the testicle are incapable of protecting allografts from destruction in previously immunized hosts.[17] Lending further credence to the status of the thymus as a privileged site was our finding that intrathymic islet allografts did not sensitize their hosts to subsequent donor strain test skin grafts, which were rejected with normal tempo. This outcome is consistent with in-

TABLE 3. *Survival of Lewis Islets in WF Rats Inoculated with Syngeneic Spleen Cells from Intrathymic Lewis Islet Recipients*

Status of WF Spleen Cell Donor	Treatment of Recipient*	Days of Islet Allograft Survival
Intrathymic Lewis islets†	450R	8, 9, 12
Naive	450R	12, 14, 16

* Rats were irradiated on day −2 and received 250 to 300 × 10^6 spleen cells on day −1 relative to islet transplantation.

† Animals had retained intrathymic Lewis islet allografts for more than 100 days.

terruption of the afferent arc of the immune response, which is also a characteristic of most other privileged sites.

While the prolonged survival of intrathymic islets in naive hosts may be explained by the immunologically privileged status of the thymus, the finding that recipients bearing established intrathymic grafts are unable to reject subsequent donor strain islets suggests that mechanisms that alter systemic alloimmune responses are also contributory. We have previously demonstrated that frequencies of cytotoxic T-cell precursors specific for donor alloantigens are reduced in intrathymic islet-bearing hosts.[4] Thus one such mechanism may involve deletion or functional inactivation of alloreactive clones by the foreign cells within the thymus. Nevertheless the possibility remains that additional mechanisms such as generation of suppressor or anti-idiotypic T cells may contribute to the acceptance of extrathymic grafts. In fact suppressor cell-mediated unresponsiveness has been shown to develop in several models of long-term graft survival, particularly those in which acceptance of the allograft is achieved by administration of potent immunosuppressive agents.[25,26] Our finding that islet allograft survival in secondary hosts was not prolonged by transfer of lymphoid cells from intrathymic islet-bearing recipients argues against the generation of regulatory cell populations as the basis for the survival of intrathymic islets and further supports the conclusion that the unresponsive state to extrathymic grafts is mediated primarily by deletion or inactivation of specific T-cell clones within the thymus. That skin allograft survival was not prolonged by the intrathymic islets was somewhat disappointing because it indicates that the degree of unresponsiveness we have induced by this method is not comparable to that which is caused by neonatal inoculation of donor-strain bone marrow, and thus should probably not be referred to as "tolerance."[27]

A central focus of the present study was to test the capacity of the thymus to protect islets transplanted to spontaneously diabetic BB rats. It is important to remember that these transplants were done in hosts that had already destroyed their native pancreatic islets. In this sense they were presensitized to islet tissue and presumably had an expanded population of effector cells reactive to putative autoantigens in the transplanted islet tissue.[28] The barrier of autoimmune damage to islets may, in fact, be at least as important to the success of islet transplantation as rejection because in both animals and humans it can result, by itself, in islet failure.[29] Viewing the experiment in this context, it is rather surprising that the outcome of intrathymic islet transplantation was so good. In fact in none of the 11 acutely diabetic BB rats transplanted with Lewis islets was there ever an indication from blood glucose values or histology that even mild rejection or autoimmune insulitis occurred. In contrast all intraportally transplanted islets were rapidly and completely destroyed. In addition to these controls, an extensive collective experience exists from our previous work and that of others concerning the fate of islets transplanted to other extrathymic sites in BB rats (reviewed in Markmann et al.[29]). We and others have noted that transplanted islets are destroyed by BB rats even when the contribution of allogeneic rejection is eliminated by pretransplant *in vitro* culture or by the induction of neonatal tolerance. Thus in no previous reports, despite the fact that BB rats are relatively immunodeficient, could failure from rejection or autoimmune damage be consistently prevented unless islets were subjected to pretransplant culture or unless heavy or continuous immunosuppression was used. For example, when Selawry et al.[30,31] transplanted islets to a privileged site (the testicle) of BB rats, they found it necessary to use either pretransplant tissue culture or ALS (or both) to obtain prolonged survival. Neither pretransplant culture or immunosuppression was used at any stage in the experiments in BB rats reported here.

The possibility that autoimmune destruction of islets is an MHC-restricted process raises another interesting question: Will MHC-compatible islets fare worse than MHC-incompatible ones in BB rats because MHC restriction might protect the latter from autoimmune destruction?[12] Regardless of this consideration, we found the intrathymic site to be protective because both uncultured WF and Lewis islets implanted there uniformly survived while those transplanted to the renal subcapsule or liver did not.

While the prolonged survival of intrathymic islets in BB hosts must, at least in part, be due to the immunologically privileged status of the thymus, the finding that recipients bearing established intrathymic grafts fail to destroy subsequent extrathymic islets either by rejection or autoimmunity argues that additional mechanisms that alter systemic immune responses are also involved. Although the evidence for this presented here in BB rats is good, it is not quite as firm as that in our previous report on the weakening of the alloimmune response we caused in chemically diabetic rats. In three of the four BB rats that were retransplanted with intraportal islets after prolonged residence of an intrathymic graft, the persistence of normoglycemia after removal of the thymus demonstrated that the intraportal graft was not damaged by either rejection or autoimmunity, a conclusion later confirmed histologically. In the fourth rat, however, removal of the thymus did cause hyperglycemia. Examination of the liver of this rat revealed many islets that stained only weakly for insulin granules. No mononuclear infiltrate was noted. This finding is compatible with either of the following explanations: (1) the number of islets transplanted intra-

portally was insufficient to maintain normoglycemia and the remaining beta cells degranulated; (2) there was specific autoimmune destruction of islet beta cells while the nonbeta cell populations were protected from rejection by the influence of the intrathymic allograft.

Somewhat surprisingly and in contrast to our earlier experiments in streptozotocin-diabetic WF rats, it was not necessary in BB rats to use any immunosuppression to achieve permanent survival of intrathymic islet allografts or to induce unresponsiveness to subsequent extrathymic ones. A possible explanation for this is that the profound T-cell lymphopenia characteristic of BB rats allowed intrathymic islets to survive long enough to effect unresponsiveness. However, despite the immunodeficient status of BB rats, the eventual destruction of the majority of intraportal and renal subcapsular islet allografts demonstrates that these animals retain the capacity to respond to foreign tissue grafts and further underscores the superior nature of the thymus as a transplantation site.

We previously postulated that the donor-specific unresponsive state induced by intrathymic islet transplantation develops as T lymphocytes maturing in the thymus become tolerant to the antigens present within this specialized microenvironment. In animals that are fully immune competent, this effect may require transient depletion of mature peripheral T cells to prevent destruction of the intrathymic graft. However, if prolonged allograft survival in the thymus is achieved on some basis other than immunosuppression, gradual physiologic replacement of the peripheral T-cell repertoire by newly matured T cells may produce similar results. Although evidence exists that a sizeable percentage of peripheral T cells are relatively long lived,[32] the prolonged interval between intrathymic grafting and subsequent intraportal retransplantation (more than 120 days) used in our studies of BB rats may have been sufficient for extensive repopulation of the periphery with "tolerant" lymphocytes. On the other hand, it is possible that, in contrast to our findings using ALS-treated rats, the presence of intrathymic grafts in nonimmunosuppressed hosts can lead to development of regulatory cell populations that inhibit immune responsiveness to donor alloantigens. We are currently addressing this possibility.

This report provides further evidence that the success of intrathymic transplantation of islet allografts depends on the immunologically privileged status of the thymus as well as on systemic alterations in the immune system that this approach induces. Furthermore our demonstration that intrathymic islet transplantation can restore permanent euglycemia in spontaneously diabetic BB rats indicates that the thymus can protect islets from both allogeneic rejection and destruction by recurrent anti-beta-cell autoimmunity. The applicability of this approach to the treatment of human type I (insulin-dependent) diabetes mellitus remains to be determined.

Acknowledgments

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Discussion

DR. ANTHONY P. MONACO (Boston, Massachusetts): It is a pleasure for me to discuss Dr. Naji and Dr. Barker and their group's paper. I have had the privilege of also reading it last night. This is a provocative and outstanding and highly intelligent work. It is a continuum of their extraordinary work over the years, and they never disappoint us. This work is another example of their extraordinary scientific ability and intellectuality.

In their paper today, they have demonstrated that the thymus seems to have the characteristics of a privileged site; that is, that islets placed in the thymus seem not to be rejected. In their manuscript, which I had the opportunity to read, if that experiment is done in a sensitized animal, that is, sensitized to the histocompatibility antigens of the islets placed in the thymus, the experiment does not work, and those islets are rejected.

This is characteristic of the classic privileged site phenomenon. What is different is that residence in the thymus seems to confirm some extrathymic message in the form of extrathymic tolerance. This tolerance, however, also is kind of unique. At least we do not have all the information to determine whether it is typical tolerance of the Billingham-Brent-Medalar type.

First of all when islets were transplanted later extrathymically, they were not rejected as long as islets were in the thymus. We would like to know whether this tolerance extends to similar tissues of the same histocompatibility type, such as skin or liver, or some other tissue, or whether it is unique just for the thymus.

Furthermore this phenomenon still probably needs some extra thymic immunologic ablation because, when it is done not in the BB rat, they have had to use antilymphocyte serum, and, as Drs. Naji and Barker point out in their paper for me, this BB rat has a certain amount of immunodeficiency.

So I have three questions. First have you done any experiments to see whether the tolerance extends to nonislet tissues? Secondly, and most important, if this is an exclusion of the T-cell r
ertoire of the MHC of the islet, then it should be able to be accomplished by putting any tissue of the same MHC in that thymus, like lymphocytes, splenocytes, etc.

And then thirdly, does the type of challenge that one does peripherally affect the tolerance that you get? That is if you have more of an immunogenic challenge, is the tolerance broken?

It is a pleasure for me to discuss this extraordinary paper.

DR. FRANK THOMAS (Greenville, North Carolina): Our group has also been interested in this area, and the mechanism by which a striking tolerance to islets is achieved, the subject addressed by these studies. We agree that suppressor cells are unlikely to explain this phenomenon.

Cells reactive to donor islets developed by both positive and negative selection in the thymus, an organ described as the place T cells go for their college education. As first described by Miller, Bevan, and others, a number of cells can act within the thymus to execute what has been called a veto phenomenon, effectively vetoing the antidonor activity of a developing thymic cell.

Although commonly involving T cells, the veto mechanism can involve macrophage and dendritic cells, cells acting in concert, some think even with somatic donor cells, which of course would include donor islets. Our group has identified the veto phenomenon in a model of adult tolerance we have studied, and found it to be a quite potent mechanism.

The key feature of the veto mechanism is the ability to signal developing T cells within the thymus to abrogate their programmed cytotoxicity to the donor or veto antigens. Many of the features described by Dr. Naji's group, including clonal deletion or energy as shown by MLC and CTL precursor studies, fit the veto phenomenon to a T.

The uniqueness of the thymus among body organs in generating this reaction is also quite in keeping with the veto phenomenon that occurs largely in the thymus by classical thinking.

Have you entertained the veto hypothesis to explain intrathymic-induced tolerance? There are definitive ways by which this hypothesis could be studied beyond phenotypic analysis of total thymus T-cell maturation and MLC studies performed. I wonder if you have pursued any of these studies to date?

DR. MARK A. HARDY (New York, New York): This is a most exciting and important study, and I would like to join all the others in complimenting the authors on the thorough and complete study, which I had the privilege to review last night in manuscript form.

This approach of intrathymic injection of antigen to induce immunologic unresponsiveness has really a much broader implication in transplantation than just for islet transplantation, as our own work now has shown that the use of intrathymic UVB-irradiated splenocytes permits indefinite cardiac allograft survival.

Another recent paper in *Lancet*, using isolated glomeruli injected intrathymically in a rat model, allowed subsequent donor-type kidney transplant survival in these rats.

It is therefore conceivable that the use of the approach described by the authors may permit specific clinical manipulation of the host to allow prolonged survival of allografts other than islets and thus avoid or at least reduce the toxic side effects of immunosuppression. To be clinically practical, however, the interval between the intrathymic injection and the subsequent grafting must be very short, of the order of a day or two, to adhere to the way that we preserve organs today.

Therefore I would like to ask the authors whether they have tried to shorten the interval between injection and grafting. The interval that they have used is about 100 days. In our experiments, we have used an interval of a week. This question has to be addressed very carefully.

Have they tried this approach in larger animals, as we are looking for a clinical application?

Theoretically what do they think is the role of the donor antigen-presenting cells *versus* the intrathymic antigen-presenting cells in the development of this type of nonself- and self-recognition?

What are the kinetics of the allograft unresponsiveness in this model? Specifically have they removed the thymus at any point before subsequent grafting to see whether the beneficial effect continues? Have they fulfilled in that sense Koch's postulate?

And finally I would like to ask, do they think that the organ-specific antigens play a role in induction of specific organ unresponsiveness?

I enjoyed reviewing this paper. I think it is one of the most exciting and important contributions, at least in the last year, to the field of transplantation. And I look forward to much more work on this subject in the future.

I want to thank the society for the privilege of the floor.

DR. JOSEPH E. MURRAY (Boston, Massachusetts): I join the others in acknowledging this superb experimental model. I have always been intrigued by the lag time in many aspects of scientific progress. In this instance there is a 19-year interval from the original description by Raviola and Karnovsky of a blood–thymic barrier in the thymic cortical capillaries to its use in experimental models. As soon as I return home, I will look up that original paper to find out just why they were pursuing that particular phase of thymic anatomy.

This time lag is reminiscent of the lag between Lillie's 1916 observation of the placental intermingling of blood in freemartin cattle and the 1945 report of Ray Owens on the immunologic consequences of this anatomic anomaly. Owens' report led directly to the Billingham, Brent, Medawar paper in *Nature* in 1953 on "Actively Acquired Immunological Tolerance in Mice," a foundation piece in transplantation biology, for which Medawar received a Nobel Prize.

Raviola and Karnovsky apparently traced the lymphatics in the thymus and found absence of the efferent component. Like Dr. Monaco I am intuitively attracted to all possibilities in transplantation that might eliminate the need for immunosuppressive drugs. There are hints that naturally occurring immunosuppressive agents may exist. Dr. John Mannick and James Mobray published on such a serum factor years ago.

Sir Roy Calne noted 20 years ago that the pig liver seemed to possess a natural immune tolerance. Currently his group in Cambridge, England have reported in rats that the serum from successfully allografted liver recipients is protective when tranfused into untreated liver recipients. This observation reminds me of Nate Kaliss's enhancing antibody, which protected tumors from being rejected. Recently a Canadian group has reported that human intestinal allografts seem to survive and function better in the presence of a liver allograft. These observations may be clues for the future.

You state in your manuscript that your animals are not tolerant in the Billingham, Brent, Medawar sense. Billingham, Brent, and Medawar used to wonder whether they were producing tolerant cells or tolerant animals. How would you answer that question today on the basis of your current experience?

Finally I was surprised that the renal capsule was partially a privileged site. I enjoyed this paper very much not only for its clinical but also for its biologic implications. The model applications may well become a classic.

DR. JAMES C. THOMPSON (Galveston, Texas): I thought I might break into this Transplantation Mafia here. My ignorance of the thymus amounts to a national resource, but I would like to ask the authors about duration of thymus activity. We know that the thymus gradually stops working after a while; and at what age does it lose this ability to effect this kind of magic transmutation?

And if that has been studied, is there any way to prolong this immune protective function of the thymus, stick in a new one, or anything like that?

DR. JOHN S. NAJARIAN (Minneapolis, Minnesota): The authors have described an intriguing possibility regarding the formation of some form of immunologic tolerance. They have demonstrated that this is an organ-specific phenomenon, because skin graft from a donor of the same strain is rejected.

I did not get a chance to read the manuscript, but I am intrigued by the immunologic question it raises. That is if you implant islets into the thymus, remove the thymus containing the islets, and then challenge the host with islets from a rat of the same strain, do the second islets survive? In other words do you have cellular tolerance to that specific organ? If so that is the first report I have seen of immunologic tolerance in which persistent antigen has not been present.

And so this is my final question: Have you done this experiment and does it work? When you remove the thymus containing the islets, will a second transplant of islets from the same strain survive?

DR. ALI NAJI: Dr. Murray, the origin for the idea of intrathymic cellular transplantation is the classic paper of Billingham, Brent, and Medawar in *Nature* (172:603, 1953). In this landmark report, which I have reread many times, the authors described the concept and the feasibility of induction of neonatal tolerance in mice inoculated with cells of lymphoid and nonlymphoid tissues such as testes and kidneys. Curiously the total number of animals rendered tolerant was actually small. One wonders if the inefficiency of the tolerance-inducing inoculum was due to the proportion of lymphoid and nonlymphoid cells or to the technical difficulty of targeting the cells to the fetus. It is well known that neonatal inoculation of cells of lymphohematopoietic origin routinely produces a high degree of tolerance. We reasoned that the failure of nonlymphoid cells to induce tolerance may have been related to inability of such cells to "home" to the thymus, whereas cells of lymphohematopoietic origin would be expected to migrate to the thymic microenvironment. Thus we initiated experiments to assess whether cells of nonlymphoid origin if implanted in the microenvironment of the thymus would be capable of inducing tolerance.

We have found that the acquisition of peripheral immune unresponsiveness after intrathymic islet inoculation permits indefinite survival of extrathymic islets, but only slight prolongation of skin allografts. It appears that the thymus behaves as a classic immunologically privileged site and is subject to the usual biologic characteristics of such sites, in that prior sensitization of the host with skin allografts precludes prolonged survival of intrathymic islets.

The question of why occasional islet allografts survive permanently in the renal subcapsule without a prior intrathymic implant is intriguing. The duration of these islet survival was variable, however, and on long-term observation they were all rejected. In contrast virtually all intrathymic islets were protected against rejection and produce a remarkably superior degree of blood glucose homeostasis than intraportal or subrenal capsule islet allografts.

Dr. Najarian, you asked whether the peripheral unresponsiveness could be maintained after the removal of the thymus bearing the alloantigen. We have not carried out extrathymic islet transplants after removal of the thymus, but to some extent our work in the BB rats addresses your question. In our experiments the spontaneously diabetic BB rats were transplanted with intrathymic islets and approximately 100 days later were challenged with an extrathymic (intraportal) allogeneic islets. The BB recipients harboring both intrathymic and intrahepatic islet allografts were observed for an additional 2 months before removal of the thymus bearing the islet allografts. In thymectomized hosts observation for an additional month showed no destruction of the extrathymic islets or recurrence of diabetes. Therefore it appears that the continued presence of the thymus bearing allogeneic islets is not necessary for maintenance of the peripheral unresponsiveness.

Dr. Thompson asked whether the involution of the thymus with age influences the survival of the intrathymic islets. We were initially concerned that with the progressive involution of the thymus we might observe destruction of the islets and recurrence of diabetes. The recipients in our studies were all adults, however, and transplants were carried out at 10 to 12 weeks of age. We have now followed other intrathymic islet recipients for more than 2 years, a period close to the life span of the rat, without any recurrent diabetes.

Dr. Hardy, we are delighted that Dr. Remuzzi and his colleagues from Italy (*Lancet* 337:750, 1991) have confirmed our work by their recent report of prolonged survival of renal allografts after intrathymic glomerular transplantation.

The question of the requirements of the donor *versus* recipient antigen-presenting cells is an important one. We have addressed this issue by intrathymic transplantation of *in vitro* cultured islets to delete intra-islet APCs. This strategy permits us to dissect the contribution of islet endocrine cells (expressing class I major histocompatibility complex [MHC] antigens) or the intra-islet antigen-presenting cells (expressing both class I and class II MHC antigens) toward induction of peripheral unresponsiveness. Our preliminary data indicate that APC-depleted intrathymic allografts survive indefinitely. Interestingly APC depletion obviates the need for administration of a single dose of antilymphocyte serum to reduce the peripheral mature T lymphocytes. We have not assessed the

precursor frequencies of the cytologic T lymphocyte (CTL) or helper T cells to assess the tolerogenic potential of class I or class II MHC antigen-bearing cells, however.

Dr. Thomas's remarks were related to the veto phenomenon as a mechanism for the induction of peripheral unresponsiveness. The veto phenomenon as described by Fink and Bevan assumes an interaction between two lymphoid cell populations. I am not aware that nonlymphoid cells, such as islet endocrine cells, can participate in the veto network phenomenon. We have assumed that the peripheral unresponsiveness is due to the persistence of the alloantigen-bearing parenchymal cells (islet endocrine cells) in the thymic microenvironment and their influence on maturing thymocytes. There is evidence that maturing thymocytes are more susceptible to tolerance-inducing signals and moreover that "inappropriate" presentation of antigen by nonlymphoid cells induce a state of anergy in T cells.

Dr. Monaco asked about the abolition of the tolerance by reconstitution of the hosts with syngeneic cells. As you know this constitutes a classic method to abolish neonatal tolerance, and we have begun to assess the impact of the syngeneic lymphoid cell reconstitution on abolition of tolerance after intrathymic islet transplantation. We have carried out further studies on the efficacy of intrathymic transplantation of cells of lymphohematopoietic origin to induce peripheral unresponsiveness. Our preliminary results are encouraging, in that intrathymic inoculation of lymphohematopoietic cells possessing tolerogenic potential appears to induce peripheral unresponsiveness toward other tissue and vascularized organ allografts.

LATTA.002C4 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Latta, Paul P.
Appl. No.	:	10/823,263
Filed	:	April 13, 2004
For	:	A METHOD OF TREATMENT OF DIABETES THROUGH INDUCTION OF IMMUNOLOGICAL TOLERANCE (as amended)
Examiner	:	Belyavski, Michail A.
Group Art Unit	:	1644

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on **May 6, 2005.**

Daniel Altman

Daniel Altman, Reg. No. 34,115

AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to Office Action mailed January 26, 2005, and interview conducted March 21, 2005 please amend the above-identified application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Summary of Interview begins on page 5 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace the Title of the Invention with the following title:

A METHOD OF TREATMENT OF DIABETES THROUGH INDUCTION OF IMMUNOLOGICAL TOLERANCE

AMENDMENTS TO THE CLAIMS

1. **(Currently amended)** A method of treating diabetes in a mammal in need thereof, comprising the steps of:

implanting in said mammal a tolerizing dose of insulin-secreting cells ~~from the same species as said mammal~~ encapsulated in a biologically compatible permselective membrane; then

administering to said mammal a ~~curative~~ therapeutic dose of corresponding unencapsulated insulin-secreting cells.

2. **(Original)** The method of claim 1, wherein said mammal is a human, canine or feline.

3. **(Currently amended)** The method of claim 1, wherein said tolerizing dose is one to two orders of magnitude less than said ~~curative~~ therapeutic dose.

4. **(Original)** The method of claim 1, wherein said insulin-secreting cells are pancreatic islet cells.

5. **(Original)** The method of claim 1, wherein said membrane comprises polyethylene glycol.

6. **(Currently amended)** The method of claim 1, wherein said tolerizing and ~~curative~~ therapeutic doses ~~are~~ comprise porcine cells.

7. **(Currently amended)** The method of claim 1, further comprising the step of administering one or more anti-inflammatory agents to said mammal prior to, at the same time as, or subsequent to administration of said ~~curative~~ therapeutic dose.

8. **(Original)** The method of claim 1, wherein said membrane has a molecular weight cutoff of about 150 kDa or less.

9. **(Original)** The method of claim 1, wherein said membrane has a pore size of less than about 0.4 μm.

10. **(Original)** The method of Claim 9, wherein said membrane has a pore size of less than about 0.2 μm.

11. **(Currently amended)** The method of Claim 1, wherein said ~~curative~~ therapeutic dose is between one and two orders of ~~magniture~~ magnitude higher than said tolerizing dose.

12. **(Original)** The method of Claim 1, wherein said implanting step is subcapsular, subcutaneous, intraperitoneal or intraportal.

13. **(Original)** The method of Claim 1, wherein said administering step is intraperitoneal, intraportal or subcutaneous.

14. **(Original)** The method of Claim 1, wherein said tolerizing dose is administered incrementally.

SUMMARY OF INTERVIEW

Exhibits and/or Demonstrations

Experimental data showing that implanting a tolerizing (sub-therapeutic) dose of encapsulated insulin-producing cells into NOD mice prior to the animals developing diabetes protected these animals from diabetes for the rest of their natural lives as shown by their normoglycemia and lack of insulitis.

Identification of Claims Discussed

1-14

Identification of Prior Art Discussed

USP 6,703,017; 6,425,764; Posselt et al. Diabetes 1992 41:771-775.

Proposed Amendments

None

Principal Arguments and Other Matters

The Applicant argued that claims 1-14 are non-obvious over USP 6,703,017 and 6,425,764 in view of Posselt et al. *Diabetes* 1992 **41**:771-775.

Results of Interview

Applicant will provide Declaration showing the histology of the mice that were prevented from developing Type I diabetes. Applicant agreed to consider amending claims. The Examiner provided the correct copy of the Posselt et al. reference (i.e. 1991 *An. Surg.* **214**:363-373).

REMARKS

Claims 1-14 are pending. Claims 1, 3, 6, 7, and 11 have been amended. Support for the amendments can be found in the Specification as filed, for example, 8:30-31 and 9:26-27. The following addresses the substance of the Office Action and the Examiner Interview.

1. Title of the invention is not descriptive

The Examiner has requested amending the Title of the Invention to clearly indicate the invention to which the claims are directed. Applicant has now amended the Title accordingly.

2. References in IDS not found in File

Applicant has resubmitted the references previously submitted in the parent application in Applicant's co-pending Application No. 10/660,924 of which the present application is a continuation. Accordingly, pursuant to 37 C.F.R. 1.98(d), additional copies of the references are not submitted in the present application.

3. Compliance with 35 USC §112, second paragraph

The Examiner has rejected Claims 1-14 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. More specifically, the examiner has stated that there is insufficient antecedent basis for the limitation "curative" in the claim. During the interview on March 21, 2005, the Examiner indicated that the use of the term "therapeutic" would be acceptable. Applicant has now amended claims 1, 3, 6, 7, and 11 to recite the term "therapeutic" instead of "curative". Support for this amendment can be found on page 8, line 30-31 of the Specification as filed. Claim 6 was additionally rejected as being indefinite and ambiguous in the recitation of "wherein said tolerizing and curative doses are porcine", because "doses" can not be porcine. Applicant has amended claim 6 to read "wherein said tolerizing and therapeutic doses comprise porcine cells." Support for this amendment can be found in the Specification as filed, page 9, lines 26-27. Therefore, Claims 1-14 are now in compliance with 35 USC §112, second paragraph.

4. Compliance with 35 USC §112, first paragraph

The Examiner has rejected Claims 1-14 under 35 U.S.C. 112, first paragraph, as being not in compliance with the enablement requirement. The Examiner stated that the specification does not reasonably provide enablement for a method of treating Type I diabetes in a mammal

comprising implanting a tolerizing dose of insulin-producing cells encapsulated in a biologically compatible membrane and then administering a curative dose of corresponding unencapsulated insulin-secreting cells. According to MPEP 2164:

> "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." A patent need not teach, and preferably omits, what is well known in the art."(2164.01)
>
> There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. "not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted. All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art."

Here, the scope of the claims is a method of inducing tolerance to insulin-producing cells implanted in an animal, including human, for the therapeutic result of treating diabetes. Every element of claim 1 is well within the level of knowledge of a skilled artisan, e.g., implanting foreign cells is common practice by medical doctors specializing in transplants; encapsulating methods are also well-known to a person skilled in the art, and Patent 5,529,914 which has been incorporated by reference into the Specification as filed teaches methods of encapsulating cells for implantation; calculating tolerizing dose and therapeutic dose for a specific mammal is disclosed in Examples 1 to 7 as "one or two orders of magnitude less than a curative dose". These examples give the tolerizing dose and therapeutic dose for several important medical conditions as follows:

Disease	Cells	Tolerizing Dose	Curative (Therapeutic) Dose	Support in the Specification
Diabetes	Islets/insulin producing cells	100-2,000 islets/kg of body weight 1,500 islets/kg of body weight	10,000-20,000 islets/kg of body weight 15,000 islets/kg of body weight	12:26-30 25:13-16
Parkinson's	Adrenal Chromaffin cells	1,000 cells/kg of body weight	10,000 cells/kg of body weight	25:21-26
Hemophilia	Liver cells	2,500 cells/kg of body weight	5,000 cells/kg of body weight	25:29-26:5

Disease	Cells	Tolerizing Dose	Curative (Therapeutic) Dose	Support in the Specification
Liver Transplant	Liver cells	1,000 cells/kg of body weight	Whole liver	26:7-11
Myasthenia gravis	Neural cells expressing acetylcholine receptor	2,500 cells/kg of body weight		26:13-19
General		Between about 100 cells/kg body weight and about 5,000 cells/kg body weight	Between about one and two orders of magnitude higher than tolerizing dose	13:1-4

Based on the guidance provided, a person skilled in the art would immediately know the dose required for their patient depending on the condition to be treated. Medical doctors routinely calculate dosages for patients by considering such factors, including but not limited to, weight, age, sex, degree of disease, etc. A curative (therapeutic) dose of implanted islets is well known in the field and a person skilled in the arts could readily calculate a one to two magnitude decrease in this dose to obtain the numbers for the tolerizing dose.

The specification also provides experimental proof of principle, i.e. experimental data showing that an encapsulated insulin-producing cells given as a small mass insufficient by itself to induce normoglycemia permits a second, unencapsulated, implant of insulin-producing cells in a therapeutic dose to survive as shown by normalized blood glucose levels in the treated mice in which diabetes had been induced by intravenous injection of streptozotocin. See Example 1.

Nevertheless, the Examiner is questioning the value of such evidence based on alleged lack of predictability of the treatment of diabetes in human from *in vivo* data obtained in murine models of diabetes. However, the Examiner is setting forth a much stricter standard than required by law. MPEP 2107.03 establishes the following:

> Evidence does not have to be in the form of data from an art-recognized animal model for the particular disease or disease condition to which the asserted utility relates. Data from any test that the applicant reasonably correlates to the asserted utility should be evaluated substantively. Thus, an applicant may provide data generated using a particular animal model with an appropriate explanation as to why that data supports the asserted utility. The absence of a certification that the test in question is an industry-accepted model is not dispositive of whether data from an animal model is in fact relevant to the asserted utility. Thus, if one skilled in the art would accept the animal tests as being reasonably predictive of utility in humans, evidence from those tests should be considered sufficient to

support the credibility of the asserted utility."

In the Declaration by David Scharp, M.D. submitted under 37 CFR §1,132, additional data is presented which shows that the claimed method works in two murine models of diabetes: the streptozotocin-induced diabetic mice and in non-obese diabetic (NOD) mice. These are the two well-known and widely accepted murine models of diabetes. The multiple low-dose streptozotocin (MLDS) model of diabetes is characterized by progressive hyperglycemia and insulitis similar to that observed in recent onset type I diabetics (Like and Rossini, 1976 *Science* **193**:415-417). The NOD mouse model also shares clinical serological and histo-immunological features with human type I diabetes (Bach, 1994 *Endocrine Rev.* **15**:516-542). As in humans, the disease is characterized by infiltration of the pancreatic islets by immune cells, insulitis followed by destruction of the β-cells. Both models have been used extensively to study new therapies for diabetes. In fact, the NIH recognize the NOD mouse as THE model animal for diabetes and maintains a research colony and data base on these animals for researchers. The NIH state "The NOD mouse, which spontaneously develops type 1 diabetes, is a valuable animal model that is used extensively in research exploring the etiology, prevention, and treatment of this disease. It is a vital research tool for testing promising prevention and treatment strategies at the preclinical level." (http://www.niaid.nih.gov/dait/NODmice.htm, copy attached herein).

The evidence in the Declaration reiterates the results provided in the specification showing that the claimed method is effective to permit survival of a therapeutic dose of insulin-producing cells and thereby effectively treating diabetes. *See* Declaration ¶ 6. Moreover, the Declaration also establishes that implantation of a sub-therapeutic, tolerizing dose of insulin-secreting cells is effective to create immunological tolerance to insulin-secreting cells, namely the host's own islet cells. Together these results establish that the claimed method of treating diabetes by tolerizing the host immune system prior to implanting the fully therapeutic dose of the insulin-producing cells works to permit the host to receive the fully therapeutic dose without rejection. *See* Declaration ¶¶ 7-16.

Therefore, using the proper standard set forth in the MPEP, the evidence provided by Applicant in the Specification and in the Declaration submitted herewith clearly supports that one skilled in the art would accept the murine models as reasonably correlating to the condition in human.

The Examiner also believed that undue experimentation would be required to determine screening and testing protocols. However, as is apparent from the claim, the goal of the invention is to treat diabetes, i.e. achieve and maintain normoglycemia. Methods for determining whether normoglycemia is present have been exceedingly well known for many years; only routine blood glucose monitoring would be required to demonstrate the efficacy of the claimed invention.

5. Compliance with 35 U.S.C. 103(a)

The Examiner has rejected Claims 1-4 and 6-14 under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425,764, or US Patent 5,629,194 each in view Posselt et al. (1991 *Ann. Surg.* 214:363-373). Pursuant to MPEP 2143, in order to establish a *prima facie* case of obviousness three requirements must be met: First there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. In the case of the present invention, the cited references fail to suggest all of the claim limitations.

The '017 patent describes implanting insulin-producing cells in a dose of about 8,000-12,000 islets/kg of patient's body weight (col. 14, lines 7-9) to create a pancreas-like structure in a human patient. Therefore, the implant in USP '017 is designed to treat diabetes by creating a live "insulin pump" in the body. Furthermore, Example 12 of USP '017 describes implanting 5,000 islets per NOD mouse (this dose equals 200,000 islet/kg of body weight), which resulted in normoglycemia in these animals. The '017 patent does not teach or suggest implanting a dose of insulin-producing cells encapsulated in a biologically-compatible membrane prior to implanting the fully-therapeutic dose, wherein the tolerizing dose is at least one order of magnitude less than the therapeutic dose.

US Patent 5,425,764 describes a method of using an implantable bioartificial pancreas device containing insulin-secreting islets, to supply an exogenous source of insulin to treat the symptoms of diabetes. Accordingly, the '764 patent requires implantation of a therapeutic dose of insulin-secreting cells, i.e. the dose necessary to achieve normoglycemia. As such, the '764 Patent does not describe or suggest implanting a tolerizing dose of insulin-producing cells

encapsulated in a biologically-compatible membrane prior to implanting of fully therapeutic non-encapsulated dose, where the tolerizing dose is at least one order of magnitude less than the therapeutic dose.

US Patent 5,629,194 describes a method of implanting embryonic porcine pancreatic non-insulin-secreting cells capable of proliferating *in vivo* and then secreting insulin after transplantation. The dose sufficient for the treatment of insufficient insulin activity is 100,000-500,000 aggregates, each containing 300-500 cells per human patient. This is a fully therapeutic dose. Thus, the '194 patent does not describe or suggest implanting a tolerizing dose of insulin-producing cells encapsulated in a biologically-compatible membrane prior to implanting of fully therapeutic non-encapsulated dose, where the tolerizing dose is at least one order of magnitude less than the therapeutic dose.

Posselt et al. describes implanting unencapsulated islets into various areas of the body, liver, kidney, and thymus, of spontaneously diabetic BB rats. The only implantation site that showed survival of the implanted cells was the thymus. The intrathymic islet recipients were observed for a period close to the life span of the rat, without any recurrent diabetes. In additional experiments, approximately 100 days after the initial intrathymus transplantation, the transplanted rats were challenged with an extrathymic allogeneic islets, which remained intact even after removal of the thymus bearing the islet allografts. However, as the authors stated several times in this article, thymus is considered to be an immunologically privileged site and is subject to the usual biologic characteristics of such sites, in that prior sensitization of the host with skin allografts precludes prolonged survival of intrathymic islets (page 272, right column). The experiments, performed by Posselt et al. show just that, i.e. when allogeneic islets were transplanted into the thymus of recipients that had previously rejected donor strain skin grafts, the islets were destroyed in an accelerated manner, demonstrating that the intrathymic site is readily accessible to activated T cells (page 367 left column, and page 368, right column), and that no tolerance can be achieved using this protocol. Furthermore, Posselt et al. goes on stating that the achieved tolerization to intrathymic allografts is due to their direct influence on maturing thymocytes, which are more susceptible to tolerance-inducing signals, and that such "inappropriate" presentation of antigen by nonlymphoid cells induce a state of anergy in T cells (page 373). Therefore, Posselt et al. teaches away from using tolerizing dose of insulin-producing cells anywhere but thymus, and it does not teach encapsulating these cells.

Furthermore, as Dr. Scharp states in his Declaration submitted herewith, the BB rat has multitude of immunologic disorders that makes it more of a model for immune deficiency than for diabetes. Therefore, the BB rat is no longer considered an acceptable model for studying human autoimmune diabetes. This is also stated by Posselt et al.: "BB rats are known to be significantly immunodeficient" (see page 365, left column, line 5-6).

Here, the instant method is not limited to any specific implantation site for the tolerizing dose of encapsulated cells and still ensures survival of subsequently implanted un-encapsulated cells. Furthermore, opposite to Posselt et al., it works even in models where the immune system has already been sensitized.

Therefore, none of the cited references suggest the claimed limitation that a tolerizing dose is implanted prior to a therapeutic dose. Accordingly, even when combined, these references do not teach all the limitations of the claimed invention. As such, the cited references fail to support a *prima facie* case of obviousness. Therefore, Claims 1-4 and 6-14 are in compliance with 35 U.S.C. 103.

The Examiner has rejected Claim 5 under 35 U.S.C. 103(a) as being unpatentable over US Patent 6,703,017 or by US Patent 5,425,764, or US Patent 5,629,194 each in view Posselt et al. (1991 *Ann. Surg.* **214**:363-373) as applied to Claims 1-4 and 6-14, and further in view of USP 5,529,914. Non-obviousness of the independent Claim 1 in view of US Patent 6,703,017 or by US Patent 5,425,764, or US Patent 5,629,194 each in view Posselt et al. is asserted above. The US Patent 5,529,914 discloses a method of encapsulating cells, but it fails to cure the deficiencies of US Patent 6,703,017, US Patent 5,425,764, US Patent 5,629,194, and Posselt et al. Therefore, Claim 5 is in compliance with 35 USC §103(a).

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, amendments to the claims, the reasons therefor, and arguments in support of the patentability of the pending claim set are presented above. In light of the above amendments and remarks, reconsideration and withdrawal of the outstanding rejections is specifically requested. If the Examiner finds any remaining impediment to the prompt allowance of these claims that could be clarified with a telephone conference, the Examiner is respectfully requested to initiate the same with the undersigned.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 6 May 2005

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LATTA.002C4 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Latta, Paul P.
Appl. No.	:	10/823,263
Filed	:	April 13, 2004
For	:	A METHOD OF TREATMENT OF DIABETES THROUGH INDUCTION OF IMMUNOLOGICAL TOLERANCE
Examiner	:	Belyavski, Michail A.
Group Art Unit	:	1644

DECLARATION OF DAVID SCHARP, M.D.

UNDER 37 C.F.R §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Dear Sir:

1. I, David Scharp, M.D., am Executive Vice President and Chief Medical Officer of Novocell, Inc. At Novocell I am actively engaged in research related to development of treatments for diabetes using encapsulated insulin-producing cells.

2. I have extensive experience in the field of the claimed invention as indicated in the attached Curriculum Vitae provided herewith as Exhibit A.

3. Working with the sole inventor of the present application, Paul P. Latta, and others, I carried out experiments to evaluate the efficacy of treating diabetes through induction of immunological tolerance.

4. The currently available approach to treating diabetes, which does not require repeated administration of insulin, is transplantation of insulin-producing cells from a donor to the diabetic patient. However, the problem with this approach is that such transplants do not survive the attack of the host immune system unless the patients are under continuous, life-long immunosuppression.

5. The approach of the present invention is totally different in that we are implanting a very small fraction of a therapeutic dose of encapsulated islet allografts prior to implantation of the fully therapeutic dose in order to tolerize the immune system to the foreign antigens shed by these encapsulated allografts made impervious to the assault by the host immune system. The fraction of encapsulated islets in the tolerizing dose is so small it would have no effect on controlling blood glucose by itself. However, the continuous release of donor antigens from this small fraction of encapsulated islets not destroyed by the host, alters the immune response in a way that the host no longer considers the implanted islets to be foreign. The tolerization therefore, protects the later implanted non-encapsulated islet allografts from being rejected by the host, allowing them to take permanent hold in the host body and produce the fully therapeutic amount of insulin as needed to treat diabetes.

6. In our original experiments, described in the Specification of the above-identified application, we induced diabetes in mice by intravenous injection of streptozotocin. Induction of diabetes by streptozotocin injection is a well-known procedure which destroys pancreatic insulin-producing cells. We first implanted into these mice a small, sub-therapeutic dose of encapsulated insulin-producing cells. Two to three weeks later the same mice received an additional, large (therapeutic) unencapsulated implant (2,000-3,000 cell aggregates, 1000 cells per aggregate) of insulin-producing cells. We showed that the encapsulated insulin-producing cells given as a small mass of 100 cell aggregates (1,000 cells per aggregate) permits this second unencapsulated implant to survive as shown by normalized blood glucose levels in the treated mice.

7. We then turned to another animal model of diabetes, non-obese diabetic (NOD) mice, and proved that the method works in this model as well.

8. There are two available animal models for studying Type I diabetes, the BB rat and the NOD mouse. The BB rat, while developing diabetes, has a multitude of immunologic disorders that makes it more of a model for immune deficiency than diabetes. The BB rat is no longer considered an acceptable model for studying human autoimmune diabetes.

9. The NOD mouse is therefore, the only animal model for autoimmune, Type I diabetes that it is predictive of human disease. The lymphocytes of the NOD mouse spontaneously begin attacking its insulin-producing pancreatic beta cells soon after birth. Looking at the histology of the pancreas of these mice during the autoimmune process, one finds early that most islets are infiltrated with immune cells that are destroying islets. As the process continues towards the diabetic phase, almost all of the beta cells are destroyed leaving smaller than normal islets with residual inflammation that continues to destroy the new islets that are stimulated to develop due to the failing islet mass. This ongoing destruction of the insulin-producing pancreatic beta cells continues and progresses for 15 to 32 weeks until a sufficient number of beta cells are destroyed to cause the clinical onset of Type I diabetes in NOD mice. Examining living NOD mice prior to development of clinical diabetes, including monitoring their blood glucose levels, one would have no clue that this autoimmune process is actively destroying their pancreatic beta cells. Yet, if one examines their blood for anti-islet protein antibodies, one can clearly identify those animals that will eventually lose blood glucose control and develop clinical diabetes. This situation is identical for human Type I diabetes in that patients at high risk for developing Type I diabetes are tested for the presence of specific auto-antibodies. The number and titers of these specific antibodies can predict with >90% certainty which of these patients with ongoing autoimmune destruction of their beta cells will actually develop clinical Type I diabetes within 5 years.

10. We encapsulated islets from mouse strain C57Bl/6 by polyethylene glycol conformal coating as described in USP 5,529,914. This patent was incorporated by reference into the specification of the application captioned above. We then implanted these conformally-coated islets by intraperitoneal injection into NOD mice. The experiment had two variables under study

– time of implantation of the encapsulated islets (4, 8, and 12 weeks) and number of islets implanted (50, 100 and 150 islet equivalents, IEQ).

11. **Exhibit 1** shows the data for all animals grouped by time of implantation. Implantation at 4 weeks showed the best results with diabetes being prevented in 60% of the treated animals, as compared to the control animals with none having diabetes prevented.

12. **Exhibit 2** shows the data for all animals grouped by number of islets implanted. A dose of 50 IEQ produced the best results with diabetes being prevented in 60% of the treated animals. All the control animals developed diabetes.

13. **Exhibit 3** demonstrates that in the control recipients (NOD mice not implanted with encapsulated islet cells), the autoimmune destruction of the pancreatic islets is very complete with small shrunken islets remaining with continuing evidence of lymphocytes destroying any new islets that are formed.

14. **Exhibits 4 & 5** demonstrate that in those recipients that were prevented from developing diabetes after implanting the small quantity of encapsulated islets, very large islets (many times their normal size) are present, without evidence of host lymphocyte destruction. This means that the normal process in the mouse to replace lost islets has been successful to the point of preventing diabetes from destroying all of the newly formed islet cells.

15. Therefore, we have shown that: a) a small, sub-therapeutic tolerizing dose of encapsulated insulin-producing cells prevents the host immune system from attacking a later-implanted un-encapsulated therapeutic dose of insulin-producing cells; and b) a small, sub-therapeutic tolerizing dose of encapsulated insulin-producing cells prevents the host immune system from attacking the host insulin-producing cells in the pancreas.

16. Combined, these experiments prove that the claimed method of treating diabetes by tolerizing the host immune system prior to implanting the fully therapeutic dose of the insulin-producing cells works to permit the host to receive the fully therapeutic dose without rejection. This process works without the need of continuous, life-time immunosuppression of the host.

17. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: May 4, 2005 By: David W Scharp

David Scharp

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